# Modulation of the expression and function of dopaminergic presynaptic proteins by the statins : Potential implication for the therapeutic intervention in Parkinson's disease.

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Par

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Né le 19 mars 1986 à Schiltigheim

# Modulation de l'expression et de la fonction des protéines dopaminergiques présynaptiques par les statines.

## Application potentielle pour une intervention thérapeutique dans la maladie de Parkinson.

**Membres du Jury**

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| M. François DARCHEN, Directeur de recherche, CNRS UMR 8250, Paris | Président |
| M. Stéphane HUNOT, Directeur de recherche, CNRS UMR 7225, Paris | Rapporteur |
| M. Etienne HERZOG, Chargé de recherche, CNRS UMR 5297, Bordeaux | Membre invité |
| M. Francisco Javier GARCIA-LADONA, UCB Biopharma SPRL, Belgique | Membre invité |
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# Résumé

La maladie de Parkinson (MD) est caractérisée par une perte progressive des terminaisons présynaptiques dopaminergiques et reste actuellement incurable. Néanmoins, dans les études épidémiologiques, il a été montré que l'utilisation des statines, médicaments hypocholestérolémiants, diminue le risque de développer une MD. Les statines sont également capables d'inhiber les effets neurodégénératifs dans les modèles précliniques *in-vitro* et *in-vivo* de la MD. Cependant, les mécanismes moléculaires à l'origine de ces effets neuroprotecteurs ne sont pas encore complétement élucidés.

Ainsi, nous avons étudié les effets potentiels des statines sur l'expression des marqueurs synaptiques et sur le transport de la dopamine. Dans nos études, les statines induisent la croissance des neurites dans les cellules dopaminergiques et déclenchent une augmentation de l'expression des protéines synaptiques dopaminergiques telles que le transporteur vésiculaire des monoamines (VMAT2) et le transporteur de la dopamine. Les statines induisent une diminution de la recapture de la dopamine cellulaire et des changements d'affinités aux niveaux des sites de liaison des inhibiteurs sélectifs du VMAT2. L'activation du facteur de transcription nucléaire protéine-1 se liant à l'élément de régulation des stérols (SREBP-1), cholestérol-dépendent, serait l'élément inducteur de la surexpression des marqueurs dopaminergiques présynaptiques induite par les statines.

En outre, ces résultats soutiennent un potentiel thérapeutique neuroprotecteur et/ou neurorestaurateur des statines précédemment proposées dans la MD et permettent de mettre en évidence de nouvelles cibles thérapeutiques comme le facteur SREBP.

**Mots clés:** maladie de Parkinson, statine, cholestérol, dopamine, VMAT2, DAT, SREBP

# Abstract

Parkinson disease (PD) is characterized by a progressive loss of dopaminergic presynaptic terminals and remains incurable. However in epidemiological studies, it has been shown that the use of statins, which are hypocholesterolemic drugs, diminishes the risk to develop a PD. Statins are able to inhibit the neurodegenerative effects in *in-vitro* and *in-vivo* models of PD. However, the molecular mechanisms driving neuroprotective effects are not yet fully understood.

Consequently, we investigated the potential effects of statins on the synaptic expression and dopamine transport function in the dopaminergic system. In our studies, statins enhance the neurite outgrowth in the dopaminergic cells and trigger an increase in the expression levels of presynaptic dopaminergic proteins such as vesicular monoamine transporter 2 (VMAT2) and dopamine transporter. Statins induce a reduction of dopamine cellular uptake and modulate the binding-affinity of the specific inhibitors for VMAT2. The activation of the nuclear transcriptional factor sterol regulatory element-binding protein 1 (SREBP-1), cholesterol-dependent, could be the key element of the overexpression of dopaminergic presynaptic markers induced by the statins.

Furthermore, these findings highlight the therapeutic neuroprotective and/or neurorestorative potentials of statins previously proposed in PD and allow to bring out new potential therapeutic targets such as SREBP factor.

**Key words:** Parkinson's disease, statin, cholesterol, dopamine, VMAT2, DAT, SREBP

# Résumé substantiel

La maladie de Parkinson (MP) est une maladie neurodégénérative caractérisée par une perte des terminaisons présynaptiques dopaminergiques de la voie nigro-striée. Sur un plan clinique, cette maladie se traduit principalement par des symptômes moteurs tels que l'akinésie, la rigidité articulaire, l'instabilité posturale et les tremblements mais aussi par des symptômes non-moteurs. Les symptômes moteurs de la MP sont détectables lorsque près de 80% des terminaux présynaptiques sont perdus. Et, les mécanismes conduisant aux troubles neurodégénératifs et fonctionnels de la voie nigro-striée sont, à ce jour, inconnus et pourrait être multifactoriels.

Plusieurs mutations génétiques telles que l'α-synucléine (SNCA) ou kinase 2 de répétition riche en leucine (LRRK2) sont associées à la MP. Plus particulièrement, la mutation α-synucléine A53T et la duplication ou triplication des gènes sont directement impliquées dans la neurodégénérescence neuronale, perte caractéristique de la MP. Néanmoins, les formes familiales liées à une mutation représentent seulement une minorité des cas de la MP (10%) et ne peuvent pas expliquer la majorité des cas sporadiques. Les toxines environnementales ont également été identifiées comme des facteurs ou des inducteurs de la MP. Plusieurs voies et cibles synaptiques ont été désignées comme ayant une implication ou un rôle majeur dans les mécanismes neurodégénératifs de la MP. Entre autres, les protéines synaptiques et vésiculaire telles que le transporteur-2 vésiculaire des monoamines (VMAT2; SLC18A2) ou le transporteur de la dopamine (DAT; SLC6A3) sont affectées par la MP. Ces deux transporteurs jouent un rôle essentiel dans la recapture de la dopamine extracellulaire. Le DAT recapture la dopamine de la fente synaptique pour la transporter vers le cytosol. Le VMAT2 s'occupe, quant à lui, de la recapture de la dopamine cytosolique pour la stocker dans la vésicule synaptique. Cette recapture est essentielle pour assurer un bon niveau de libération de dopamine dans la fente synaptique en vue de l'activation des récepteurs dopaminergiques postsynaptiques.

La dérégulation de ces transporteurs pourrait être, en partie, la cause de la MP. Lorsque les perturbations motrices se manifestent, 70% de la dopamine et 50% des protéines impliquées dans le métabolisme de la dopamine, notamment la tyrosine hydroxylase (TH), le DAT ou le VMAT2, sont perdues dans le striatum, tandis que, seuls 30% des neurones dopaminergiques se trouvant dans la substance noire sont perdues. Ceci

implique un dysfonctionnement précoce des terminaux pré-synaptiques. En outre, plusieurs études montrent que les neurones exprimant un niveau élevé de DAT sont plus vulnérables. Les études *in-vivo et in-vitro* dans des modèles de la MP ont mis en évidence l'implication du DAT dans la recapture des agents neurotoxiques tels que le 1-méthyl-4-phényl pyridinium ($MPP^+$), le 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine (MPTP), le paraquat ou la rotenone. De manière générale, les animaux sur-exprimant le DAT présentent une neurodégénérescence. Le DAT semble également être dérégulé par les mutations présentes dans la MP. Par exemple, l'α-synucléine mutante renforce la toxicité des agents neurotoxiques dans la surexpression du DAT comparé l'α-synucléine sauvage. Concernant le VMAT2, plusieurs études génétiques ont pu établir un lien entre la MP et l'expression de VMAT2. Des mutations de VMAT2 ou de son promoteur ont des effets neuroprotecteurs ou inducteurs de la MP. Les études réalisées sur des animaux qui présentent une régulation négative ou positive de l'expression du VMAT2 suggèrent que ce dernier a un rôle dans les mécanismes de détoxication de la dopamine et des agents neurotoxiques. De plus, les animaux ayant une régulation négative de VMAT2 produisent du stress oxydatif mais présentent également des agrégations d'α-synucléine ainsi qu'une perte des neurones dopaminergiques de la voie nigro-strié. Enfin, la modulation positive du VMAT2 a été identifiée comme ayant des effets protecteurs dans les modèles de la MP parce qu'il rétablit les niveaux physiologiques de la dopamine et qu'il séquestre la dopamine oxydées et les agents neurotoxiques.

L'ensemble de ces mécanismes protectifs ou dégénératifs impliquant le DAT ou le VMAT2 dépend essentiellement du niveau de dopamine cytosolique. La dopamine est instable : elle se transforme et produit de la dopamine-o-quinone, la dopamine oxydés et des dérivés réactifs de l'oxygène. Ces métabolites sont extrêmement toxiques pour la cellule et dérégulent plusieurs métabolismes physiologiques essentiels pour la cellule comme par exemple la production d'ATP par la mitochondrie. Quand la dopamine se situe dans le milieu extracellulaire ou dans les vésicules synaptiques, elle est susceptible d'être moins toxique. Le pH acide de la vésicule entraîne une stabilisation de la dopamine qui n'est donc plus transformée en métabolites toxiques. Il en est de même pour les agents neurotoxiques : leur séquestration permet de réduire leur toxicité. De plus, une concentration élevée de dopamine au niveau de la fente synaptique peut induire une plus forte stimulation post-synaptique potentiellement bénéfique dans la MP. Par ailleurs, l'augmentation du stockage de la dopamine dans les vésicules synaptiques peut également

contribuer à une libération plus élevée de dopamine dans la fente synaptique, ce qui peut induire une plus forte stimulation postsynaptique. Néanmoins actuellement, il n'y a pas de substance capable de moduler l'activité ou l'expression à la hausse du VMAT2 et les inhibiteurs du DAT n'ont pas montré d'amélioration des symptômes dans la MP malgré des effets prometteurs dans les modèles de MP.

A ce jour, les traitements de la MP visent à la pallier le manque de dopamine soit par l'utilisation de levodopa (L-Dopa, L-3,4-dihydroxyphenylalanine), un précurseur de la dopamine, soit par des agonistes dopaminergiques. Il existe également des substances qui permettent de réduire la dégradation de la dopamine. Bien que ces substances soient efficaces pour le traitement des symptômes moteurs au début de maladie, la progression de la maladie rend ces médicaments inopérants. De plus, des effets secondaires peuvent apparaitre. Pour ces raisons et en vue d'améliorer la qualité de vie des patients, il est nécessaire de trouver de nouvelles substances pharmacologiques neuroprotectrives et neurorestauratrices des voies dopaminergiques afin de bloquer l'initiation ou d'endiguer la progression de la MP.

C'est dans ce cadre que les statines ont été identifiées comme ayant un rôle potentiel dans la neuroprotection. Elles sont des hypolipidémiants/hypocholestérolémiant qui agissent par inhibition de l'enzyme limitant la synthèse du cholestérol, autrement appelée HMG-CoA réductase (hydroxyméthylglutaryl-CoA réductase). Les analyses épidémiologiques ont mis en évidence une réduction du risque de développer la MP lors de l'utilisation des statines, ce qui suggère un potentiel thérapeutique de ces molécules.

Les données pharmacologiques actuellement disponibles ont montré que les statines sont capables d'inhiber les effets neurodégénératifs des neurotoxines telles que la 6-hydroxydopamine (6-OHDA) et le MPTP dans les cultures neuronales ainsi que dans des modèles animaux de la MP. Les effets neuroprotecteurs des statines ont été expliqués par des effets pléiotropiques tels que la réduction des réponses pro-inflammatoires (interleukines, facteur nucléaire kappa B, facteur de nécrose tumorale), la réduction des facteurs oxydatifs et le blocage de l'agrégation de l'α-synucléine. De plus, les statines pourraient induire une augmentation de l'expression de l'oxyde nitrique synthase endothéliale, enzyme qui joue un rôle neuroprotecteur par une augmentation de la circulation sanguine cérébrale et par un relâchement de l'endothélium vasculaire. Ces

effets pléiotropiques impliqueraient de nombreuses voies de signalisations comme la voie de la phosphoinositide 3-kinase(PI3K) /Akt et la voie de prenylation avec le membre 3 de la famille de gènes homologue ras (RhoA). Cependant, les mécanismes moléculaires qui conduisent à ces effets neuroprotecteurs ne sont pas encore pleinement caractérisés et compris. Par ailleurs, les statines seraient également capables, dans des cellules non-neuronales, d'augmenter l'expression des marqueurs synaptiques, à l'image de la protéine vésicule synaptique 2A (SV2A). C'est également un mécanisme qui a été très peu exploré. Des études sur les protéines effectrices de la voie de signalisation du cholestérol peuvent expliquer l'augmentation d'expression des protéines synaptiques. En effet, l'activation de SREBP (protéines se liant à l'élément de régulation des stérols) serait elle aussi capable de moduler l'expression des protéines synaptiques telles que la synaptobrevin ou le SV2A comme observé avec les statines. SREBP est un facteur nucléaire connu pour activer la transcription des enzymes de la voie du cholestérol. Son activation est sensible à la baisse du cholestérol cytoplasmique. SREBP agit comme un système rétrocontrôle pour réguler à la hausse le cholestérol quand il est bas. Par l'action inhibitrice de la synthèse du cholesterol, les statines seraient capables d'activer SREBP. Elles pourraient ainsi induire l'activation de la transcription de protéines synaptiques.

Dans le cadre de ce projet doctoral, nous avons commencé par étudier le potentiel trophique des statines et leur impact sur l'expression des marqueurs synaptiques dopaminergiques ainsi que sur les fonctions de transport de la dopamine (DA) dans les cellules dopaminergique SH-SY5Y de neuroblatome humain. Nos résultats montrent que le traitement des cellules avec les statines induit la croissance des neurites avec un effet spécifique sur leur ramification. Les statines augmentent la complexité de l'arborisation neuritique, ce qui n'est pas observable avec d'autres substances trophiques telles que l'acide rétinoïque ou avec l'inhibiteur de ROCK (Y-27632).

Ensuite, nous avons voulu vérifier si la croissance neuritique pouvait être accompagnée d'une augmentation de l'expression des marqueurs synaptiques. En effet, les statines sont capables d'augmenter les biomarqueurs dopaminergiques présynaptiques, comme le VMAT2, les vésicules synaptiques 2A et 2C (SV2C), la synaptogyrin-3 (SYNGR3), ou la TH, observation faite grâce aux méthodes d'immunocytochimie et de western blot. L'analyse de l'expression génique, mesurée par RT-PCR (réaction en chaîne de polymérase-transcriptase inverse), a confirmé une régulation à la hausse rapide des

niveaux de mRNA pour le VMAT2, le SV2C et la SYNGR3 après traitement des cellules avec les statines.

En outre, l'augmentation de l'expression des protéines dopaminergiques peut être à l'origine d'une modulation de la fonctionnalité des transporteurs de la dopamine. Nous avons donc évalué cette possibilité par la mesure de la recapture d'une dopamine radioactive dans les cellules SH-SY5Y traitées avec les statines. Pour mesurer les effets relatifs à chacun des transporteurs de la dopamine, nous avons utilisé des inhibiteurs spécifiques du VMAT2 comme la réserpine ou la tétrabénazine ainsi que du DAT comme le GBR-12935. De manière inattendue, les résultats montrent une réduction du transport de la dopamine. Mais, il semblerait aussi qu'il y ait eu des modifications pharmacologiques du VMAT2 suite au traitement des cellules avec les statines. Ces changements pharmacologiques ont également été observés dans les cellules dopaminergiques Be(2)-M17 de neuroblastome humain transfectées avec le VMAT2. Ces résultats suggèrent que les changements pharmacologiques observés avec les statines pourraient être dus à une augmentation du VMAT2.

Nous avons aussi étudié le rôle de SREBP dans l'augmentation de l'expression des protéines synaptiques. Tout d'abord, nous avons pu confirmer que les statines induisent bien la translocation du facteur de transcription SREBP à l'intérieur du noyau. Ceci renforce la possible implication des statines dans la régulation des protéines synaptique via SREBP. A l'aide d'un activateur et d'un inhibiteur spécifique de SREBP, nous avons pu confirmer la translocation nucléaire de SREBP. L'activation de SREBP a également montré qu'il est possible d'augmenter l'expression des mêmes marqueurs synaptiques que ceux induit par les statines. L'analyse par RT-PCR des cellules traitées avec l'activateur de SREBP a confirmé une augmentation de l'expression des marqueurs VMAT2, SYNGR3 et SV2A. Enfin, la répression par siRNA de l'expression de SREBP induit une diminution de l'expression du mRNA de protéine SV2C et VMAT2

Pour conclure, les résultats montrent que les statines induisent la différenciation phénotypique des cellules SH-SY5Y, caractérisée à la fois par une augmentation de la croissance des éléments synaptiques de structure ainsi que par une augmentation de l'expression des protéines de vésicules synaptiques impliquées dans la fonction du système dopaminergique. La modulation des protéines synaptiques par SREBP suggère que leur

expression est partiellement cholestérol-dépendante. Les changements observés peuvent être à l'origine des effets neuroprotecteurs dans la MP. Premièrement, l'induction de la croissance neuritique pourrait avoir des conséquences bénéfiques au niveau de la neurorestauration des projections axonales. Deuxièmement, la chute de la recapture de dopamine par le DAT peut aider à réduire le stress oxydatifs dû à l'excès de dopamine dans le cytosol. Troisièmement, la baisse de la recapture de la dopamine peut entrainer une augmentation du niveau de dopamine extracellulaire, ce qui aide ainsi à prolonger la stimulation post-synaptique. L'augmentation de l'expression de VMAT2 peut compléter les effets neuroprotecteurs en réduisant également le stress oxydatif par la recapture de la dopamine et des agents neurotoxiques dans les vésicules. Il a été démontré que la SYNGR3, une protéine vésiculaire, pourrait également potentialiser l'effet de la recapture de VMAT2. L'interaction de la SYNGR3 avec le DAT serait à l'origine de cet effet potentiateur. Enfin, SV2C serait également un modulateur du niveau de la dopamine. Des études génétiques ont d'ailleurs montré un potentiel effet neuroprotecteur de SV2C dans la MP. Dans l'ensemble, ces résultats mettent en évidence des effets pléiotropiques des statines dans le système dopaminergique et ils soutiennent le potentiel thérapeutique neuroprotecteur et neurorestaurateur des statines précédemment proposé dans la MP et permettent de mettre en évidence de nouvelles cibles thérapeutiques comme le facteur SREBP.

# Communications

**Publications**:

M. Schmitt, B. Dehay, E. Bezard, J. Garcia-Ladona. Dopaminergic Differentiation through the Transcriptional Activation of the Sterol Regulatory Element-Binding Protein-1 in neuroblastoma cell (in preparation).

M. Schmitt, B. Dehay, E. Bezard, J. Garcia-Ladona. Harnessing the Trophic and Modulatory Potential of Statins in a Dopaminergic Cell Line. Submitted to Synapse 2015.

**Presentations and posters:**

Long-Term Organotypic Hippocampal Slice Culture as a Novel Drug Discovery Platform. P. Ghisdal, N. Noel, S. Chong, M. Schmitt, C. Wolff. 2nd NewMedecines Science congress, UCB BioPharma SPRL, Braine l'Alleud, 2015, July 7th 2015.

M. Schmitt, B. Dehay, E. Bezard, J. Garcia-Ladona. Dopaminergic Differentiation through the Transcriptional Activation of the Sterol Regulatory Element-Binding Protein-1 in neuroblastoma cell. Journée de l'école doctorale, Arcachon, France, April 16th 2015.

M. Schmitt, B. Dehay, E. Bezard, J. Garcia-Ladona. Lovastatin Activates SREBP-1 Nuclear Translocation and Modulates Dopamine Transport System in SH-SY5Y Cells. Society for Neuroscience congress, Washington D.C. United State of America, November 18th 2014.

M. Schmitt, B. Dehay, E. Bezard, J. Garcia-Ladona. Statins Induce Neurite Outgrowth and Increase the Expression of Dopaminergic System-Related Synaptic Vesicle Proteins in SH-SY5Y Neuroblastoma Cells. XX World Congress on Parkinson's Disease and Related Disorders, Geneva, December 10th 2013.

M. Schmitt. Role of the Synaptic Vesicle Proteins and their Interaction Partners in the Mechanisms of Neurodegenerative Diseases. European Medicines Research Training Network, workshop presentation – AstraZeneca, Alderley Park, Cheshire, United Kingdom, February 23rd 2012.

M. Schmitt, T. Deprez, N. Pacico, J. van Eyll, M. Page, J. Garcia-Ladona. High Content Analysis Using Definiens Software Platform to Measure Neurite Outgrowth in SH-SY5Y Cells and Hippocampal Neurons in Culture. Science Advisory Board meeting, UCB Pharma SA, Bruxelles, Belgium, 2011.

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# Abbreviations

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| **5HT** | 5-hydroxytryptamine (serotonine) |
| **6-OHDA** | 6-hydroxydopamine |
| **A2A** | Adenosine 2A |
| **AADC** | Aromatic L-amino acid decarboxylase (catalyses L-Dopa in dopamine) |
| **ADCC** | Aromatic L-amino acid decarboxylase |
| **ADH** | Alcohol dehydrogenase |
| **ADP** | Adenosine diphosphate |
| **Akt** | Protein kinase B |
| **ALDH** | 3,4-dihydroxyphenylethanol |
| **AMPA** | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| **ANOVA** | Analysis of variance |
| **ApoE** | Apolipoprotein E |
| **Asc** | Ascorbate |
| **Atorva** | Atorvastatin |
| **ATP** | Adenosine triphosphate |
| **BCA** | Bicinchoninic acid |
| **BD** | Becton Dickinson |
| **BDNF** | Brain derived neurotrophic factor |
| **BH4** | Tetrahydrobiopterin |
| **BSA** | Bovine serum albumin |
| **CaMKII** | Ca2+/calmodulin-dependent protein kinase II |
| **cAMP** | Cyclic adenosine monophosphate |
| **COMT** | Catechol-O-methyl transferase |
| **COS** | CV-1 (simian) in Origin |
| **COX2** | Cyclooxygenase 2 |
| **CREB** | cAMP response element-binding protein |
| **CRMP2** | Collapsin response mediator protein |
| **D1R** | Dopamine receptor D1 |
| **D2R** | Dopamine receptor D2 |
| **DA** | Dopamine |
| **DAPI** | 4′,6-Diamidino-2-phenyindole |
| **DARPP-32** | Dopamine- and cAMP-regulated neuronal phosphoprotein |
| **DAT** | Dopamine transporter |
| **DBH** | Dopamine-β-hydroxylase |
| **DHA** | Dehydroascorbic acid |
| **DJ1** | Protein deglycase |
| **DMEM** | Dulbecco's Modified Eagle Medium |
| **DOPAC** | 3,4-dihydroxyphenylacetic acid |
| **DOPA** | 3,4-dihydroxyphenylacetaldehyde |
| **DOPET** | 3,4-dihydroxyphenylethanol |
| **DTT** | DL-Dithiothreitol |
| **$EC_{50}$** | Half maximal effective concentration |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **$E_{max}$** | Maximal effect |
| **Enk** | Enkephalin |
| **eNOS** | Endothelial nitric oxyde synthase |
| **ERK** | Extracellular signal-regulated kinases |
| **ERM** | Ezrin, radixin and moesin |
| **FBS** | Fœtal bovin serum |
| **Fluva** | Fluvastatin |
| **FOXA2** | Forkhead box protein A2 |
| **FPP** | Farnesyl-pyrophosphate |
| **FTase** | Farnesyltransferase |
| **GABA** | γ-Aminobutyric acid |
| **GAD** | Glutamate decarboxylase |
| **GCH1** | GTP cyclohydrolase |
| **GDNF** | Glial derived neurotrophic facto |
| **GDP** | Guanosine diphosphate |
| **GGPP** | Geranylgeranyl-pyrophosphate |
| **GGTase** | Geranylgeranyltransferase type 1 |
| **GLP1R** | Glucagon-like peptide 1 receptor |
| **glu** | Glutamate |
| **gp91Phox** | NOX2 / NADPH oxidase 2 |
| **GPCR** | G-protein–coupled receptor |
| **Gpe** | Globus pallidus pars externalis |
| **Gpi** | Globus pallidus pars internalis |
| **GTP** | Guanosine triphosphate |
| **HBSS** | Hank's balanced salt solution |
| **hECSs** | Human embryonic stem cells |
| **HEK** | Human Embryonic Kidney |
| **HMG-CoA** | 3-hydroxy-3-methylglutaryl-coenzyme A |
| **HMGCR** | HMG-CoA reductase |
| **HVA** | Homovanilic acid |
| **$IC_{50}$** | Half inhibition effective concentration |
| **IL** | Interleukin |
| **$I_{max}$** | Maximal inhibition |
| **iNOS** | Inducible nitric oxide synthase |
| **Insig** | Insulin-induced gene 1 protein |
| **iPS** | Induced pluripotent stem cell |
| **LDCV** | Large dense-core vesicle |
| **LDL** | Low density lipoprotein |
| **L-Dopa** | L-3,4-dihydroxyphenylalanine, levodopa |
| **LIMK** | LIM domain kinase |
| **LINGO-1** | Leucine-rich repeat and immunoglobulin domain-containing protein 1 |
| **LPS** | Lipopolysaccharide |
| **Lova** | Lovastatin |
| **LRRK2** | Leucine-rich repeat kinase 2 |
| **LXR** | Liver X receptor |

| | |
|---|---|
| **MAG** | Myelin associated glycoprotein |
| **MAO** | Monoamine oxidase |
| **MB-COMT** | Membrane-bound COMT |
| **MES 23.5** | Substantia nigra/neuroblastoma hybrid cell line |
| **MFS** | Major Facilitator Superfamily |
| **mGluR** | Metabotropic glutamate receptor |
| **MLC** | Myosin light chain phosphatase |
| **MLCP** | Myosin light chain protein |
| **MOPS** | 3-(N-morpholino)propanesulfonic acid |
| **MPP+** | 1-methyl-4-phenylpyridinium |
| **MPTP** | 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| **mRNA** | Messenger ribonucleic acid |
| **MW** | Molecular weight |
| **mβCD** | Methyl-β-cyclodextrin |
| **NEDD-4-2** | Neural precursor cell expressed developmentally downregulated protein 4 |
| **NET** | Norepinephrine transporter |
| **NF-κB** | Nuclear factor-kappa B |
| **NgR** | Nogo Receptor |
| **$n_H$** | Hill coefficient |
| **NMDA** | N-methyl-D-aspartate |
| **NO** | Nitric oxide |
| **Nogo** | Reticulon-4 |
| **NTN** | Neuturin |
| **NURR1** | Nuclear receptor related 1 protein |
| **Omgp** | Oligodendrocyte-myelin glycoprotein |
| **p75NTR** | p75 neurotrophin receptor |
| **PAH** | Phenylalanine hydroxylase |
| **PBS** | Phosphate-buffered saline |
| **PBST** | PBS-tween 20 |
| **PC12** | Pheochromocytoma 12 |
| **PCR** | Polymerase chain reaction |
| **PD** | Parkinson's disease |
| **PDL** | Poly-D-lysine |
| **PET** | Positron emission tomography |
| **PFA** | Paraformaldehyde |
| **PI3K** | Phosphoinositide 3-kinase |
| **PINK1** | PTEN-induced putative kinase 1 |
| **PIP2** | Phosphatidylinositol 4,5-bisphosphate |
| **PIP3** | Phosphatidylinositl (3,4,5)trisphosphate |
| **PITX3** | Pituitary homeobox 3 |
| **PKA** | Protein kinase A |
| **PKC** | Protein kinase C |
| **PMA** | 12-O-tetradecanoylphorbol-13-acetate |
| **PMA** | Phorbol 12-myristate 13-acetate |
| **PNMT** | Phenylethanolamine N-methyltransferase |
| **PPAR** | Peroxisome proliferator-activated receptors |
| **Prava** | Pravastatin |
| **PSD95** | Post-synaptic density 95 |
| **PTEN** | Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase |
| **PVDF** | Polyvinylidene difluoride |
| **RA** | Retinoic acid |
| **RAR** | Retinoic acid receptor |
| **RGS** | Regulator of G-protein signalling |
| **RhoA** | Ras homolog gene family, member A |
| **ROCK** | Rho-associated protein kinase |
| **ROS** | Reactive oxygen species |
| **RPM** | Revolutions per minute |
| **RQ** | Relative quantity |
| **RSP** | Reserpine |
| **RT-PCR** | Reverse transcription polymerase chain reaction |
| **RXR** | Retinoid X receptor |
| **S1P** | site-1 protease |
| **S2P** | site-2 metalloprotease |
| **S-COMT** | Soluble COMT |
| **SAM** | S-adenosyl-l-methionine |
| **SAH** | S-adenosyl-l-homocysteine |
| **SDS** | Sodium dodecyl sulfate |
| **SERT** | Serotonin transporter |
| **shRNA** | Small hairpin RNA |
| **Simva** | Simvastatin |
| **siRNA** | Small interfering RNA |
| **SLC18A2** | Solute carrier family 18, member 2 |
| **SLC6A3** | Solute carrier family 6, member 3 |
| **SPECT** | Single-photon emission computed tomography |
| **SNAP-25** | Synaptosomal-associated protein 25 |
| **SNARE** | Soluble N-ethylmaleimide-sensitive factor activating protein receptor |
| **SNc** | Substantia nigra pars compacta |
| **SNP** | Single nucleotide polymorphism |
| **Snr** | Substantia nigra pars reticulata |
| **SREBP-1** | Sterol regulatory element-binding protein-1 |
| **SREBP-2** | Sterol regulatory element-binding protein-2 |
| **SSV** | Small synaptic vesicle |
| **STN** | Subthalamic nucleus |
| **SV2A** | Synaptic vesicle glycoprotein 2A |
| **SV2B** | Synaptic vesicle glycoprotein 2B |
| **SV2C** | Synaptic vesicle glycoprotein 2C |
| **SYNGR3** | Synaptogyrin-3 |
| **TBZ** | Tetrabenazine |
| **TH** | Tyrosine hydroxylase |
| **TNF** | Tumor necrosis factor |
| **tPA** | Tissue plasminogen activator |
| **TUJ-1** | Beta-3 tubulin |

| | |
|---|---|
| **UCHL-1** | Ubiquitin carboxy-terminal hydrolase L1 |
| **V-ATPase** | Vacuolar-type H+-ATPase |
| **VEGF** | Vascular endothelial growth factor |
| **Veh** | Vehicle |
| **VGAT** | Vesicular GABA transporter |
| **VGluT** | Vesicular glutamate transporter |
| **VMAT** | Vesicular monoamine transporter |
| **Wort** | Wortmannin |
| **WT** | Wild-type |
| **Y** | Y-27632 |

# Introduction

## Literature Review

# Introduction - Literature review

## I. Parkinson's disease

### I. 1. Generality and historical background

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain (Hassler, 1938). PD has been described for first time in 1817 in "An essay on the shaking palsy" by James Parkinson (1755-1824) (Parkinson, 1817). He reported the features of the clinical syndrome of six cases as a "paralysis agitans". Later in 19$^{th}$ century, Jean-Martin Charcot (1825-1893), also described the disease and named as "Parkinson's disease" in 1872 in honour to James Parkinson (Charcot, 1872). In 1868, Theodor Hermann Meynert (1833-1892) suggested that PD was due to a defective function of the basal ganglia (Meynert et al., 1968). Friedrich Heinrich Lewy (1885-1950) described for the first time the presence of Lewy bodies, spherical inclusions in the nucleus substantia innominata and in the dorsal nucleus of the vagus of Parkinson patients (Lewy, 1912). The neurodegenerative mechanism leading to PD was proposed by the correlation between the clinical features and the nigrostriatal dopamine loss (Ehringer and Hornykiewicz, 1960). Indeed, these hypotheses were confirmed by the ability of levodopa (L-Dopa, L-3,4-dihydroxyphenylalanine) to improve motor impairment of PD patients in 1961 (Birkmayer and Hornykiewicz, 1962). During last decades, genetic mutations, abnormal misfolded proteins, oxidative stress and mitochondrial dysfunction have identified and investigated as potential factors leading to dopaminergic cells loss. However, the aetiology of PD remains poorly understood (Davie, 2008; Beitz, 2014; Kalia and Lang, 2015).

# I. 2. Epidemiology and cost burden for society

PD is most common movement disorder affecting 1% in people over 60 years of age (de Lau and Breteler, 2006). The prevalence rates of PD vary between 18 and 418 per 100,000 people worldwide and 102 and 190 per 100,000 people in western countries (Pagonabarraga and Kulisevsky, 2012). PD prevalence increases with age and it is estimated to around 17.4 per 100,000 people between 50 to 59 years old and 93.1 per 100,000 people between 70 to 79 years old (Bower et al., 1999). However, the prevalence is estimated to be around 13.4 per 100,000 people per year and the average age of onset established at around 60 years old (Van Den Eeden et al., 2003). The annual incidence rates of PD range from 4.9 to 26 per 100,000 people (Jankovic and Tolosa, 2015). The longitudinal epidemiological study in Rochester (Minnesota) reported an impact of age on the annual incidence rate, from 0.8 to 114.7 to 304.8 per 100,000 people in age group from 0 to 29, 50 to 99 and 80 to 99 years old respectively (Bower et al., 1999). In total, approximately 4.1 and 4.5 million people are affected worldwide (Dorsey et al., 2007) and 177,095 people in France (Gustavsson et al., 2011). Sometimes, epidemiological studies reported a male preponderance for PD. The PD is approximately twice more frequent in men than in age-matched women, which could suggest an X-linked genetic predisposition, influence of sex hormones or greater exposure to a causative environmental factor (de Lau and Breteler, 2006).

The mortality in PD patients was 2.9 times higher than in the general population (de Lau and Breteler, 2006; Morgan et al., 2014). But, since the introduction of L-Dopa, PD patients realized remarkable symptomatic benefits, which means that L-Dopa normalized the mortality rates to approximately 1.5 (de Lau and Breteler, 2006; Morgan et al., 2014). The long L-Dopa treatment over 12 years, the severity of Parkinson's symptoms and the appearance of dementia increase mortality risk in PD (de Lau and Breteler, 2006; Morgan et al., 2014).

In France, the economic burden is estimated at 1.26 billion of euros with an expense of 7,128 euros by patient (Gustavsson et al., 2011). The total cost for United States is projected to be 23 billions of dollars annually with an expense of 10,349 dollars by patient (Huse et al., 2005). The increase number of PD patients and their disability represents an important challenge for health care delivery systems worldwide.

# I. 3. Pathologic features and clinical diagnosis of Parkinson's disease

## I. 3.1. Motor symptoms

PD produces severe motor impairments and a subsequent decline of the quality of life over time (Kalia and Lang, 2015). PD associated symptoms have been classified in two groups: the motor symptoms and non-motor symptoms. Motor disorders associated to PD are also named cardinal signs (Pápai and Pax, 1690; Koller and Montgomery, 1997). Cardinal signs include the four most common manifestations described in PD patients: tremor, bradykinesia (akinesia), rigidity, and postural instability.

**Akinesia** or **bradykinesia** correspond respectively to a lack of movement and slowness of movement (Wilson, 1925). Bradykinesia is observed early after disease onset and corresponds to a deficit of planning, initiation and execution of movement with a reduction of its amplitude up to a complete cessation (Berardelli et al., 2001; Rodriguez-Oroz et al., 2009). A significant delay appears between the desire to realize the movement and its execution. The reductions of spontaneous movements, blinking rate, facial expressions and of arm swinging are typical manifestations of bradykinesia (Berardelli et al., 2001; Rodriguez-Oroz et al., 2009). Bradykinesia/Akinesia is considered as one of the most disabling PD symptom, severely affecting daily activities such as writing, eating or washing.

**Rigidity** is caused by an increased muscle tone leading to excessive and continuous contraction (Delwaide et al., 1986). The resistance to palpation at rest, reduced distension to passive movement, increase in the stretching-resistance and facilitation of the shortening reaction characterize this symptom. Rigidity may be associated with pain, and painful shoulder is one of the most frequent initial manifestations (Jankovic, 2008; Rodriguez-Oroz et al., 2009).

**Resting tremor** is the easily recognized symptom of PD which is characterized by a 4-6hz frequency at rest (Stanley-Jones, 1956). These tremors usually begin unilaterally with a predominant affection at the extremities of the upper limbs especially in the fingers, giving the known "pill-rolling" tremor.

**Postural instability** is due to a loss of postural reflexes and appears at the late stage of the disease (Bloem, 1992). It occurs after many years of disease progression and after onset of other clinical features. Postural instability is manifested by balance disorders which causes more and more frequent falls and contributes significantly to increase the risk of hip fractures (Jankovic, 2008; Rodriguez-Oroz et al., 2009). This is probably the most bothersome symptom because this can compromise the mobility and can become hazardous.

These four cardinal symptoms are found in different proportions depending on the patients (Jankovic, 2008). The variation of intensity of these symptoms over time can be observable in patients and depends of the personal factors such as stress, emotion, hearing and visual stimulus (Jankovic, 2008; Rodriguez-Oroz et al., 2009).

### I. 3.2. Non-motor symptoms

Non-motor complications in PD represents a major source of deterioration in quality of life and contribute to severe disability (Chaudhuri et al., 2006). Non-motor symptoms become more evident with disease progression, but they could be already present years or decades before the apparition of motor symptoms (listed in Table 1) (Chaudhuri et al., 2006). Thus, the identification of non-motor symptoms such as rapid eye movement behaviour disorder, hyposmia, constipation, sleep disorders and depression could be useful for diagnosis at early stages of the disease (Jankovic, 2008; Massano and Bhatia, 2012). Mild cognitive impairment is apparent at early stages of the disease in many cases and recent data have shown that dementia will occur in 80% of patients after 20 years of disease progression (Meireles and Massano, 2012). The neurobiological basis of non-motor symptoms are not yet fully understood, however the loss of dopaminergic, noradrenergic and / or cholinergic stimulations appear to be involved in non-motor symptoms (Wishart and Macphee, 2011). Finally, some motor and non-motor symptoms occurring in PD such as dyskinesia and hallucinations are rather linked to the side effects of PD treatments such as L-Dopa (Foster and Hoffer, 2004).

| Table 1. Non-motor symptoms in Parkinson's disease | |
|---|---|
| **Neuropsychiatric features** | Impairment to dementia (80% of the PD patients)<br>Mood disorders, especially depression (45% of the PD patients, preclinical symptom)<br>Hallucinations, illusions, delusions<br>Cognitive deterioration, ranging from mild<br>Apathy (preclinical symptom)<br>Anxiety, panic attacks (preclinical symptom) |
| **Dysautonomia** | Orthostatic hypotension<br>Constipation (80% of the PD patients, preclinical symptom)<br>Urinary dysfunction<br>Sexual dysfunction<br>Excessive sweating<br>Seborrhoea<br>Sialorrhea (i.e., drooling, also attributable to decreased swallowing movements) |
| **Sleep disorders** | Insomnia<br>Rapid eye movement (REM) behaviour disorder (30% of the PD patients, preclinical characteristic)<br>Restless legs syndrome (preclinical characteristic)<br>Periodic limb movements in sleep<br>Excessive daytime sleepiness |
| **Sensory dysfunction** | Hyposmia (i.e., loss of sense of smell) (80% of the PD patients, preclinical characteristic)<br>Decreased visual contrast and colour discrimination<br>Decreased visual motion perception<br>Abnormal sensations (paresthesias) |
| **Pain** | |
| **Fatigue** | (preclinical symptom) |
| (Anderson, 2004; Chaudhuri et al., 2006; Bonnet et al., 2012) | |

## I. 3.3. Clinical diagnosis

A diagnostic marker or test for idiopathic PD has not been developed yet (Chahine and Stern, 2011). In contrast, genetic tests allow to detect the mutated genes in the genetic forms of PD. The definitive diagnosis of PD is only achieved at the autopsy of the brain with the identification by histopathological detection of the nigrostriatal dopaminergic neurons loss and the presence of Lewy bodies or intra-neuronal inclusions. Intense research in the areas of imaging and biochemical analysis of body fluids may help to detect PD in early stages. Some exclusion and inclusion criteria (Table 2) as well as rating scales have been used for the clinimetric assessment of the motor and non-motor symptoms and disability in PD (Perlmutter, 2009) which may allow to differentiate PD from other parkinsonian disorders (Quinn, 1997; Perlmutter, 2009). Originally published in 1967, the Hoehn and Yars scale is universally accepted as a staging system measurement due to its reliability and convergent validity in assessment of PD diagnosis (Hoehn and Yahr, 1967; Goetz et al., 2004; Zhao et al., 2010). This scale is intended to reflect the degree of disease progression, and combines features of motor impairment and disability (Table 3). However, the most well-established and used scale to follow the longitudinal course of PD progression in the clinical trial is the Unified Parkinson's Disease Rating Scale (UPDRS), designed and improved by the Movement Disorder Society (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003). It incorporates elements from the pre-existing scales to provide a reliable monitoring of PD-related disability and impairment.

| Table 2. The UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria | |
|---|---|
| **Parkinson's Disease Criteria** | Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude or repetitive actions) |
| | And at least one of the following:<br>Muscular rigidity<br>4–6 Hz rest tremor<br>Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction |
| **Parkinson's disease exclusion criteria** | Familiar parkinsonism<br>Persisting strictly unilateral parkinsonism<br>Sustained remission<br>Levodopa-unresponsiveness<br>Strokes with stepwise progressive parkinsonism<br>Repeated head injuries<br>Preceding neuroleptic treatment<br>Supranuclear gaze palsy or oculogyric crises<br>Early severe autonomic, cerebellar and/or pyramidal signs<br>Early Alzheimer-type dementia<br>Cerebral tumor, communicating hydrocephalus<br>MPTP intoxication |
| **Supportive positive criteria of Parkinson's Disease** | Unilateral onset<br>Progressive disorder<br>Clinical course > 10 years<br>Persistent asymmetry affecting side of onset<br>Excellent or persistent levodopa-responsiveness<br>Levodopa-induced dyskinesias<br>Rest tremor |
| (Hughes et al., 1992) | |

| Table 3. Hoehn and Yahr scale | |
|---|---|
| A commonly used system for describing how the symptoms of Parkinson's disease progression. It was originally published in 1967 and later on modified with the addition of stages 1.5 and 2.5 to help describe the intermediate course of the disease<br>(Hoehn and Yahr, 1967; Goetz et al., 2004; Zhao et al., 2010). | |
| **Stage** | **Hoehn and Yahr Scale modified** |
| 1 | Unilateral involvement only |
| 1.5 | Unilateral and axial involvement |
| 2 | Bilateral involvement without impairment of balance |
| 2.5 | Mild lateral disease with recovery on pull test |
| 3 | Mild or moderate bilateral disease; some postural instability; physically independent |
| 4 | Severe disability; still able to walk or stand unassisted |
| 5 | Wheelchair bound or bedridden unless aided |

# I. 4. Physiopathology and anatomo-functional dysregulation in Parkinson's disease

## I. 4.1. Anatomical pathways

The main neurobiological characteristic of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta clearly detectable by using immunohistochemical methods which evidence the loss of cell pigmentation due to the neuromelanin, a metabolite of dopamine (Kastner et al., 1992). The dopamine is widely distributed in the central nervous system while the dopaminergic neurons represent less than 1% of total number of neurons (Gaven et al., 2014). Several brain pathways use the dopaminergic neurotransmission. Indeed, the dopaminergic system is composed by four major pathways located in the midbrain: nigrostriatal pathway, tuberoinfundibular pathway and mesocorticolimbic projections with mesolimbic pathway and mesocortical pathway (Beaulieu and Gainetdinov, 2011).

The most important pathway in the pathophysiology of PD is the nigrostriatal pathway, particularly involved in the production of movements (Fuxe et al., 2006). Nigrostriatal pathway involves two projection bundles from the substantia nigra pars compacta which innervate the basal ganglia including the caudate nucleus, the putamen and the specific regions of the striatum (Fuxe et al., 2006). The neurodegeneration of this pathway is clearly associated to the PD.

The mesolimbic pathway involved in the addiction and reward processes includes the ventral tegmental area of the midbrain which is connected to the nucleus accumbens of the ventral striatum (Alcaro et al., 2007).

The mesocortical pathway is implicated in cognitive control, motivation and emotional responses and involves the ventral tegmental area and its projections to the cortex (Adinoff, 2004).

The tuberoinfundibular pathway controls the hormonal secretion such as the prolactin of the pituitary glands which is activated by the dopaminergic transmission from the tuberal hypothalamus (Grattan, 2015).

## I. 4.2. Basal ganglia circuitry and Parkinson's disease

Normal voluntary movements have their origin in the activity of the motor cortex (Campbell et al., 1905; Rizzolatti and Luppino, 2001; Chouinard and Paus, 2006). Most of neurons in the motor cortex project their axons to the spinal cord, which controls the transmission of neural signals between the brain and the rest of the body. Motor cortex involves various regions such as the primary motor cortex, the premotor area, the supplementary motor area and the prefrontal and parietal associative cortex (Rizzolatti and Luppino, 2001; Chouinard and Paus, 2006). A set of cortico-subcortical loops of the basal ganglia regulates the activity of these cortical areas (Figure 1). The basal ganglia are a large group of subcortical nuclei in the forebrain and midbrain which control movements by their interconnection with motor cortex, but, also with the thalamus and the brainstem, as well as several other brain structures (e.g. limbic system) (Lanciego et al., 2012). Basal ganglia are composed by four main nuclei: the striatum (putamen, caudate and nucleus accumbens), pallidum (globus pallidus pars internalis and externalis), subthalamic nucleus and substantia nigra (pars compacta and reticulata) (Lanciego et al., 2012). The striatum is the central input structure of the basal ganglia which receives the massive excitatory glutamatergic afferents from the motor cortical regions but also from other brain regions (e.g. serotoninergic fibers from the raphe nuclei or cholinergic fibers from the pedunculopontine nucleus) (Tarsy et al., 2003).

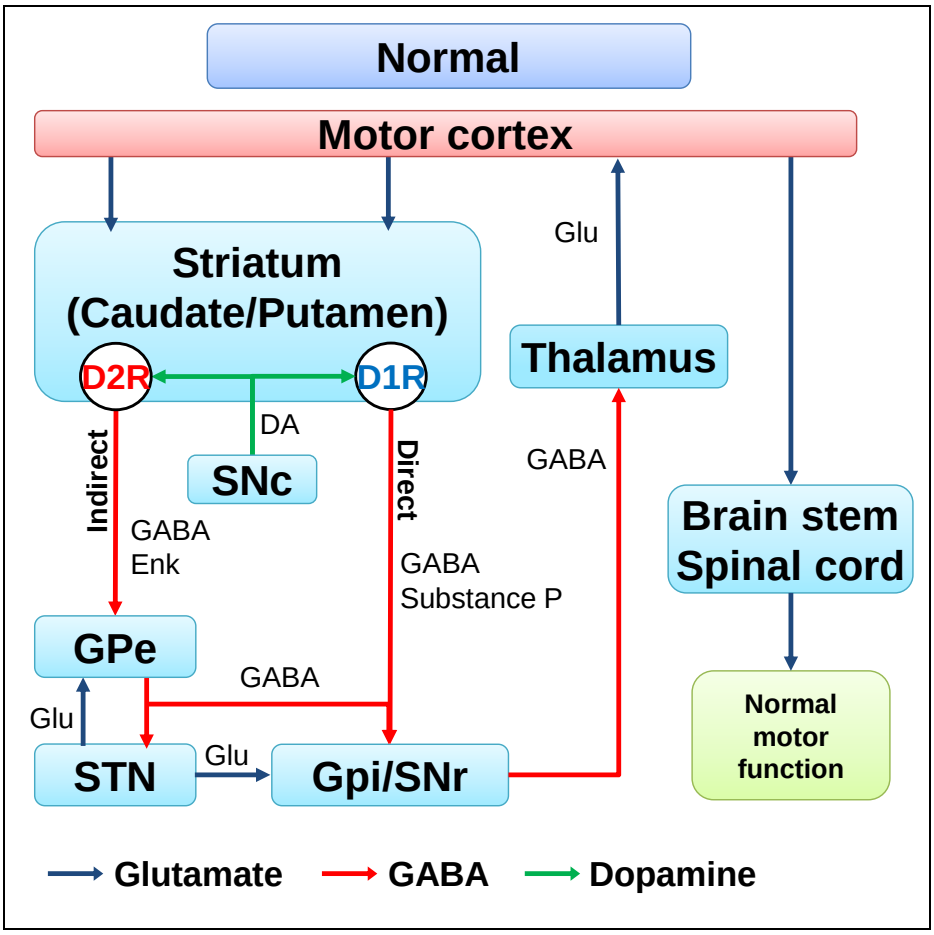


Figure 1. **Representation of a simplified schematic diagram of basal ganglia circuitry**

Glutamatergic excitatory connections, GABAergic inhibitory connections and dopaminergic connections are indicated by blue, red and green arrows respectively; Enk, enkephalin; GABA, γ-aminobutyrique acid; DA, dopamine; Glu, glutamate; GPe, globus pallidus pars externalis; GPi, globus pallidus pars interna; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus;

The neurons of the striatum could be classified as interneurons and medium spiny neurons. The interneurons representing 5% of total striatal neurons have large size (up to 60µm) and could be phenotypically grouped as cholinergic, GABAergic (γ-aminobutyrique acid), somatostatin and neuropeptide Y neurons (DiFiglia et al., 1976; Bennett and Bolam, 1994; Kawaguchi et al., 1995). Medium spiny neurons represent 95% of striatal neurons having large and extensive dendritic trees with small spines receiving synaptic inputs (Ariano, 1983; Bolam and Smith, 1990; Tepper and Bolam, 2004). They are the efferent neurons from striatum and express the GABA inhibitory neurotransmitter. We can distinguish two populations of medium spiny neuron. The first group expresses the substance P, the dynorphin, and the dopamine receptor D1 (D1R) (Alexander and Crutcher, 1990; Bolam and

Smith, 1990), whereas the second group expresses the enkephalin and the dopamine receptor D2 (D2R) (Gerfen et al., 1990; Surmeier et al., 1996).

Dopamine released by neurons from the substantia nigra pars compacta acts on the D2R and D1R expressed in striatal medium spiny neurons (Dahlstroem and Fuxe, 1964). The activation of the D1R facilitates the postsynaptic activity of the neurons whereas the activation of the D2R inhibits the postsynaptic neurons (Steiner and Tseng, 2010).

The striatal neurons with D1R are associated to the so-called direct pathway. The D1R activation inhibits the globus pallidus pars internalis and substantia nigra pars reticula that in turn reduce their GABAergic inhibition on the motor thalamus (Steiner and Tseng, 2010). The consequence of the inhibition triggered by the D1R activation leads to a stimulation of motor cortex due to the increase of glutamatergic activation from the thalamus.

An important dopaminergic functional pathway so-called indirect pathway is associated to the D2R-expressing striatal neurons. This pathway is formed by multiple connexions between the globus pallidus pars externalis and the subthalamic nucleus (Steiner and Tseng, 2010). The activation of D2R striatal neurons reduces the GABAergic inhibition on the globus pallidus externalis, which in turn causes an activation of the GABAergic inhibitory signal on the subthalamic nucleus. Thus when D2R is activated, the subthalamic nucleus activity is downregulated, which reduces the glutamatergic stimulation activity on the globus pallidus pars internalis and the substantia nigra pars reticula (Steiner and Tseng, 2010). The consequence of this neuronal cascade is a reduction of inhibition of the thalamus by the globus pallidus pars internalis and the substantia nigra pars reticula.

Both activation of D1R direct and D2R indirect pathways by the substantia nigra pars compacta reduce the inhibitory activity of the globus pallidus pars internalis on the thalamus. Subsequently, this reduction of inhibition facilitates the glutamatergic stimulation from the thalamus on the motor cortex, which triggers the motor action (Steiner and Tseng, 2010).

The loss of neuron in the substantia nigra par compacta occurring in PD disrupts the dopamine homeostasis in the basal ganglia network (Albin et al., 1989; DeLong, 1990). In this context, the degeneration of nigrostriatal pathway results in a reduction of the D1R and D2R stimulation (Figure 2) (Blandini et al., 2000; Obeso et al., 2008). The consequence of

this degeneration is a hyperactivity of globus pallidus pars internalis because, in one hand, there is less inhibition of the globus pallidus pars internalis from striatal D2R neurons, and, on the other hand, there are more discharges from subthalamic nucleus on the globus pallidus pars internalis (Blandini et al., 2000; Obeso et al., 2008). Thus, the globus pallidus pars internalis hyperactivity reduces the thalamus activity on the motor cortex. In conclusion, the loss of dopaminergic neurons in PD reduces the activity of the direct pathway associated to a hyperactivation of the indirect pathway. Combined, they lead to a deficit of the initiation of voluntary movement.

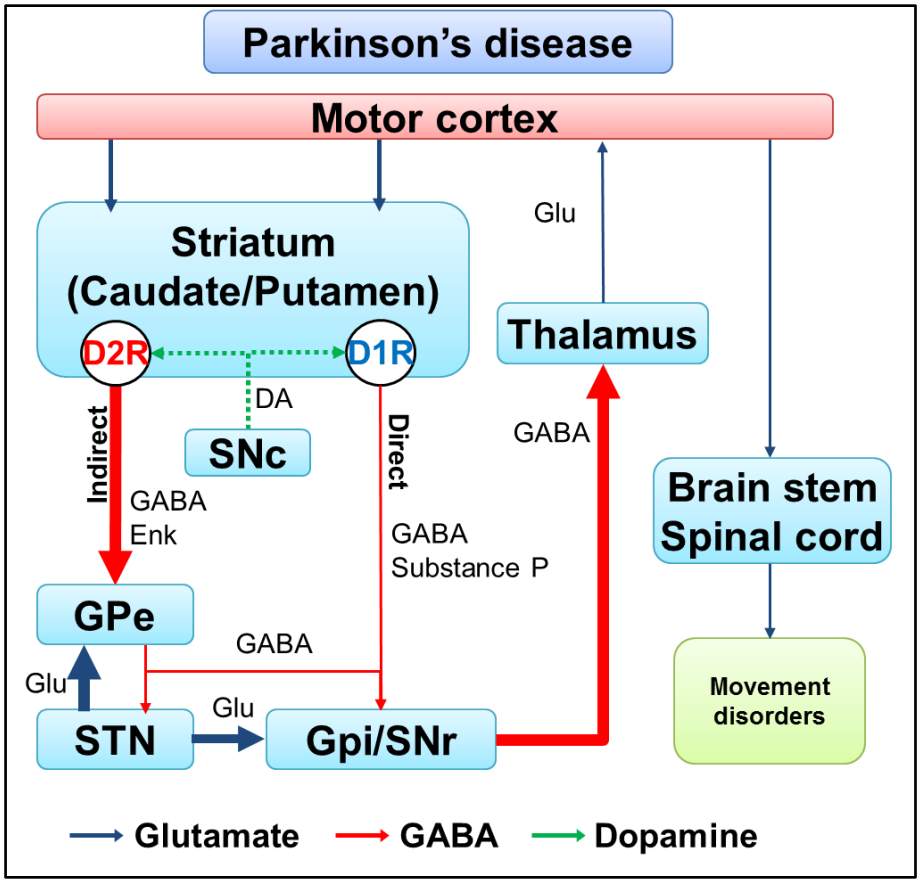


Figure 2. **Representation of a simplified schematic diagram of basal ganglia circuitry in Parkinson's disease**

Glutamatergic excitatory connections, GABAergic inhibitory connections and dopaminergic connections are indicated by blue, red and green arrows respectively; Enk, enkephalin; GABA, γ-aminobutyrique acid; DA, dopamine; Glu, glutamate; GPe, globus pallidus pars externalis; GPi, globus pallidus pars internalis; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus (Smith et al., 2012).

## I. 4.3. Alterations in other neurotransmitter systems

Although the alteration of the dopaminergic system represents the hallmark in PD, other pathways and systems are also involved. Among others, serotoninergic, cholinergic, glutamatergic and adrenergic systems in different brain areas are also impacted. The degeneration of the norepinephrine-containing neurons from the locus coeruleus of PD patients may reach 80% of the normal system, this particularly in late stage of the disease (Cash et al., 1987; Zarow et al., 2003; Del Tredici and Braak, 2013). The serotonergic cell loss in the raphe nucleus can reach up to 40% (Halliday et al., 1990; Fox et al., 2009; Politis and Loane, 2011). Both noradrenergic and serotonergic depletions could contribute to the observed non-motor symptoms disorders such as depression. Loss of cholinergic neurons reaching 50% observed in the basal nucleus of Meynert and in pedunculopontine nucleus of PD patients may be involved in axial and postural disorders (Jellinger, 1991; Pahapill and Lozano, 2000; Müller and Bohnen, 2013). Moreover, cortical lesions have also been described in late stage of PD: they could participate in the pathophysiological process (Hanganu et al., 2013).

## I. 4.4. Lewy bodies

Lewy bodies are, together with the loss of dopaminergic neurons, an important histopathological hallmark of PD (Figure 3). Lewy bodies were firstly described by Friedrich Heinrich Lewy in 1942 which suggested that "pathological changes were widespread over the whole central nervous system" (Shults, 2006). They are neuronal inclusions composed of abnormal accumulation composed about 70 different proteins including α-synuclein (Leverenz et al., 2007). PD is described in the group of synucleinopathies which also includes multiple system atrophy and dementia with Lewy bodies (Sikorska et al., 2007). Lewy bodies appear as circular intracytoplasmic inclusions (a diameter of 5–25 μm) with a dense eosinophilic core and a clearer surrounding halo (Spillantini et al., 1998; Goedert, 1999). Ultrastructurally, the Lewy body core is composed by filamentous and granular material that is surrounded by radially oriented filaments. This observation suggests an alteration of the cell cytoskeleton and of the protein degradation, which could underlie an injury of the proteasomal and lysosomal systems (Spillantini et al., 1998; Goedert, 1999). Currently, it is unknown whether Lewy bodies sequestrate the toxic misfolded proteins for cell defence or they are directly involved in the neurodegenerative process (Wakabayashi et al., 2007). Typically, Lewy body distribution follows the monoaminergic systems in the brain but their

presence is also found in many brain structures from the medulla to motor and sensory cortex. This special distribution led to the proposal of various stages for PD progression (Table 4) (Braak et al., 2002, 2003; Wolters et al., 2014). The Braak's theory is based on the observation of progressive accumulation of α-synuclein gradually extending to different brain regions. The topographic localization of Lewy bodies allowed distinguishing six stages of PD progression (Braak et al., 2002, 2003; Wolters et al., 2014).

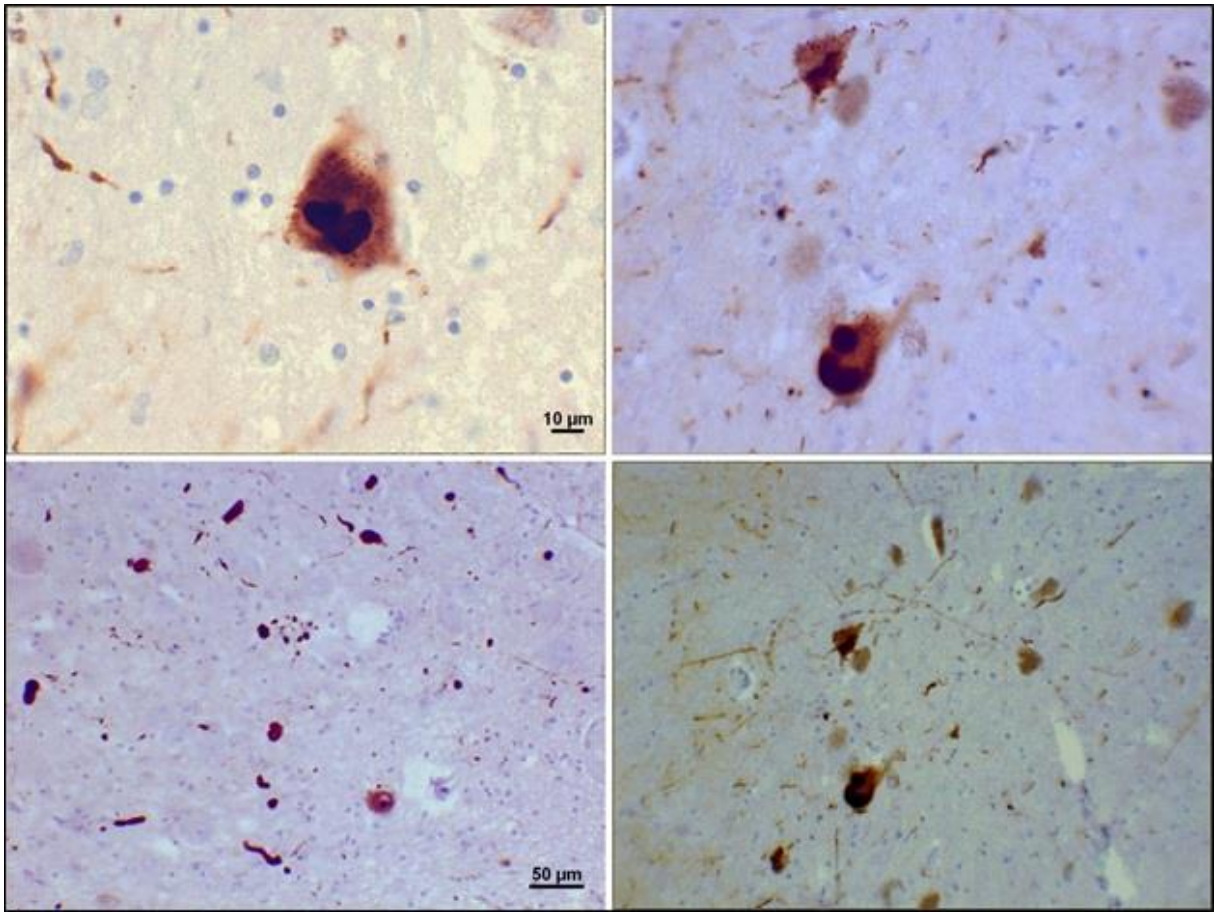


Figure 3. **Microscopic images of substantia nigra Lewy bodies and Lewy neurites in a Parkinson's patient**

A and B: Intraneuronal inclusions with α-synuclein aggregated (60x).
C and D: Lewy neurites and rounded Lewy bodies (20x).
Counterstained with Mayer's haematoxylin.
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**Table 4. Braak staging of Lewy pathology in Parkinson's disease**

The Braak model proposes that Lewy pathology progresses temporally and spatially through the following stages (Braak et al., 2002, 2003; Wolters et al., 2014)

| Braak stages | Equivalent Hoehn and Yahrs scale | Disease progression | Clinical features | General brain region | Specific brain region | Age (Years) |
|---|---|---|---|---|---|---|
| **1** | **-** | Preclinical stages: appearance of Lewy bodies | Olfactory dysfunction<br>Gastrointestinal symptom<br>Autonomic dysfunction | Medulla oblongata | Olfactory bulb<br>Dorsal nucleus of vagus nerve<br>Anterior olfactory nucleus | **50** |
| **2** | **-** | | REM sleep disorder<br>Depression<br>Anxiety | Medulla oblongata<br>Pontine tegmentum (Brain stem) | Magnocellular portions,<br>Locus coeruleus Raphe nuclei | **55** |
| **3** | **1** | Clinical stages: diagnosis | Unilateral cardinal motor signs<br>Pain<br>Perception and emotions disorders | Midbrain | Substantia nigra pars compacta<br>Pedunculopontine nucleus<br>Raphe nuclie<br>Basal nucleus of Meynert<br>Amygdala<br>Hypothalamus | **60** |
| **4** | **2** | | Bilateral cardinal motor signs<br>Psychiatric symptoms | Basal prosencephalon<br>Mesocortex | Hypothalamus<br>Thalamus<br>Mesocortex<br>Hippocampus<br>Limbic system | **65** |
| **5** | **3** | Advanced Stages: maximum spreading | Cognitive decline | Neocortex | Striatum<br>Neocortex | **70** |
| **6** | **4 - 5** | | Dementia Hospitalisation | Neocortex | Premotor<br>Motor and sensory fields | **80** |

# I. 5. Etiology

## I. 5.1. Genetic factors and familial Parkinsonism

Many genetic and environmental factors have been investigated and associated to the initiation and progression of PD. A general consensus has been reached suggesting that PD is likely caused by a combination of genetic and environmental factors and not by one single factor (Tanner et al., 1999; de Lau and Breteler, 2006). The genetic forms of PD only explain a small proportion (10%) of all PD cases that are in vast majority idiopathic forms (Massano and Bhatia, 2012). Familial Parkinsonism has been described and linked to 21 loci classified as PARK and several additional gene mutations are considered as risk factors (Table 5). For most of them, the mutation effects leading to the neurodegenerative process are not fully understood and still under evaluation. Indeed, the significance of a few PARKs in PD (PARK11, 12 and 13) remains controversial or sometimes without confirmation (replication study) (Wolters et al., 2014). However taking in account the potential cell dysfunctions associated to above mutated proteins, PD related loci could be classified in four subgroups:

**1. Protein misfolding:** PARK1 and PARK4 are caused by the mutated gene of α-synuclein protein (Polymeropoulos et al., 1996, 1997). Mutated α-synucleins may contribute to PD pathogenesis because they form a native unfolded protein and because they have the capacity to self-aggregate, which disrupts the cellular homeostasis and subsequently leads to cell death (Chauhan and Jeans, 2015).

**2. Dysfunction of ubiquitin proteasome system**: E3 ubiquitin-protein ligase (parkin, PARK2) and ubiquitin carboxyl-terminal esterase L1 (UCHL-1, PARK5) are both ubiquitously expressed in brain and involved in the ubiquitin proteasome system (Leroy et al., 1998; Paisán-Ruíz et al., 2004; Lim and Tan, 2007; Li et al., 2014). They trigger signalling cascades for the recycling and degradation of proteins. Leucine-rich repeat kinase 2 (LRRK2, PARK8) is widely expressed in human tissue and its mutation is the most common form of inherited autosomal dominant PD mutation. LRRK2 is involved in the phosphorylation cascades of the several cellular signalling pathways and more specifically in the cytoskeletal dynamic, macroautophagy and chaperone-mediated autophagy.

**3. Lysosomal dysfunction**: ATPase type 13A2 mutation (ATP13A2, PARK 9) and glucocerebrosidase (GBA) have been proposed to be involved in the dysfunction of lysosomal autophagy which plays a role in the biomolecule degradation via the intralysosomal hydrolytic enzymes (Goker-Alpan et al., 2004; Ramirez et al., 2006; Manzoni and Lewis, 2013). The impairment of autophagy-lysosomal pathways is increasingly regarded as a major pathogenic event in PD.

**4. Dysfunction of mitochondria and mitophagy**: PTEN-induced putative kinase 1 (PINK1, PARK6) and protein deglycase (DJ-1, PARK7) could play a role in the integrity and mitophagy process of the mitochondria (Valente et al., 2001; van Duijn et al., 2001; Ryan et al., 2015). The mitophagy is involved in the turnover and the maintenance of a healthy pool of mitochondria, which contributes to reduce oxidative stress. Additionally, DJ-1 modulates the gene transcription and has chaperone-like function in mitochondria. The impairment of mitochondrial function increases the oxidative stress, which leads to cell damage and cell death (Ryan et al., 2015). F-box protein 7 (FBXO7, PARK15) and its interactions with parkin (PARK2) and PINK (PARK6) were suggested for the regulation of the mitophagy (Shojaee et al., 2008; Burchell et al., 2013).

**Table 5. Clinicogenetics – Genes and loci related to Parkinson's disease**

| Locus | Typical age at onset | Chromosomal Location | Prevalence in familial PD | Mutations | Gene | Protein | Putative protein function | Inheritance | Dopa responsive | Lewy Bodies | Confirmed genes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PARK1** | EOPD | 4q22.1 | Rare 1% | Point mutations: A53T, A30P E46K, A18T, etc. | **SNCA** | α-synuclein | Unknown protein function<br>α-synuclein misfolding and aggregation | AD | + | + | + |
| **PARK2** | EOPD | 6q26 | Most common AR form: 1.4-8.2% | More than 200 mutations | **PRKN** | E3 ubiquitin protein ligase (Parkin) | Ubiquitin-protein ligase<br>Involved in UPS for protein degradation | AR | + | +/- | + |
| **PARK3** | LOPD | 2p13 | Rare | Point mutations | **SPR** | n/d | n/d | AD | + | + | + |
| **PARK4** | EOPD | 4q22.1 | Rare 1% | Duplication/ Triplication | **SNCA** | α-synuclein | Unknown protein function<br>α-synuclein misfolding and aggregation | AD | + | + | + |
| **PARK5** | LOPD | 4p14 | Extremely rare | Deletion or point mutations | **UCHL1** | Ubiquitin carboxyl-terminal esterase L1 | Hydrolyse small C-terminal adducts of ubiquitin<br>Involved in UPS for protein degradation | AD | + | - | |
| **PARK6** | EOPD | 1p36.12 | Rare: 3.7% | More than 40 mutations | **PINK1** | PTEN-induced putative kinase 1 | Mitochondrial kinase serine/threonine | AR | + | +/- | + |
| **PARK7** | EOPD | 1p36 | Rare: 2-8% | More than 10 mutations | **DJ-1** | Protein deglycase DJ-1 | Oxidative stress protection | AR | + | - | + |
| **PARK8** | LOPD | 12p12 | Most common AD form : 5 -10% | Point mutations: most common G2019S + 6 other mutations | **LRRK2** | Leucine-rich repeat kinase 2 /dardarin | Phosphorylation<br>Cytoskeletal dynamics<br>Chaperone-mediated autophagy<br>Kinase and GTPase function | AD | + | +/- | + |
| **PARK9** | JOPD | 1p36 | Rare | Point mutations and duplication | **ATP13A2** | ATPase type 13A2 | Lysosomal protein<br>Putative transport substrate function in lysosome | AR | + | - | + |
| **PARK10** | LOPD | 1p32 | Rare | Point mutations | n/d | n/d | n/d | - | + | + | |
| **PARK11** | LOPD | 2q37.1 | Rare | Point mutations | **GIGYF2** | GRB10 interacting GYF protein 2 | Regulate tyrosine kinase receptor, Regulation insulin signalling<br>Putative regulation of autophagy | AD | + | + | + |
| **PARK12** | LOPD | Xq21-q25 | Rare | Point mutations | n/d | n/d | n/d | X-linked | + | - | |
| **PARK13** | LOPD | 2p12 | Rare | Point mutations | **OMI/ HTRA2** | High temperature requirement A2 mitochondrial protein | Serine protease stimulates apoptosis by caspase activity | AR | + | + | |
| **PARK14** | EOPD | 22q13.1 | Rare | Point mutations | **PLA2G6** | Phospholipase A2 | Catalyze hydrolysis of phospholipid<br>Release of arachidonic acid and fatty acids | AR | + | + | + |

| Locus | Typical age at onset | Chromosomal Location | Prevalence in familial PD | Mutations | Gene | Protein | Putative protein function | Inheritance | L-Dopa responsive | Lewy Bodies | Confirmed genes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PARK15** | EOPD | 22q12.3 | Rare | Point mutations | **FBXO7** | F-box protein 7 | Phosphorylation dependent-ubiquitination Regulation of mitophagy | AR | + | + | + |
| **PARK16** | LOPD | 1q32 | Rare | Point mutations | n/d | n/d | n/d | AR | + | - | |
| **PARK17** | LOPD | 16q11.2 | Extremely rare: 0.08–0.14% | Point mutation: Asp620Asn, Gly51Ser, Leu774Met | **VPS35** | Vacuolar protein sorting 35 | Transport between endosomes and trans-golgi network | AD | + | - | + |
| **PARK18** | LOPD | 3q27.1 | Extremely rare: 0.02–0.2% | Point mutation: Gly686Cys, Ser1164Arg | **EIF4G1** | Eukaryotic translation initiation factor 4-gamma | Recruitment of mRNA to the ribosome | AD | + | + | |
| **PARK19** | JOPD | 1p31.3 | Extremely rare | Point mutation p.Q734X | **DNAJC6** | DnaJ (Hsp40) Homolog, Subfamily C, Member 6 (Auxilin) | Regulates the molecular chaperone activity by stimulating ATPase activity | AR | - | + | + |
| **PARK20** | EOPD | 21q22.11 | Extremely rare | Point mutation: Arg258Gln, Ala551Val | **SYNJ1** | Synaptojanin 1 | Synaptic transmission and membrane trafficking | AR | + | + | + |
| **PARK21** | LOPD | 3q22 | Extremely rare | Point mutation: Asn855Ser | **DNAJC13** | DnaJ (Hsp40) Homolog, Subfamily C, Member 13 (RME8) | Regulates the dynamics of clathrin coats in early endosomes | AD | + | + | |
| **Gaucher locus** | LOPD | 1q21 | Extremely rare | More than 200 | **GBA** | Glucocerebrosidase | Lysosomal enzyme | RF | | + | + |
| **FTDP-17** | LOPD | 17q21 | Extremely rare | 3 point mutations | **MAPT** | Tau | Stabilizes microtubules | RF | | | |
| **SCA2** | LOPD | 12q12 | Extremely rare | Point mutations and trinucleotide repeats | **ATXN2** | Ataxin-2 | Transcriptional regulation and RNA processing | RF | + | | |
| **SCA3** | LOPD | 14q32 | Extremely rare | Point mutations and trinucleotide repeats | **ATXN3** | Ataxin-3 | Involved in ubiquitin-proteasome system, deubiquitinating enzyme | RF | + | | |
| | JOPD | 5p15.33 | Extremely rare | Point mutations | **SLC6A3** | Dopamine transporter | Extracellular dopamine reuptake | AR | - | | |
| | LOPD | 2q24.1 | Extremely rare | Point mutations | **NURR1 (NR4A2)** | Nuclear receptor subfamily 4, goup A, member 2 | Nuclear receptor involves in maintenance of dopaminergic system | AD | + | + | |
| | JOPD | 11p15,5 | Extremely rare | Point mutations | **TH** | Tyrosine hydroxylase | Metabolism of dopamine | AR | + | | |

EOPD, early-onset Parkinson's disease; LOPD, late-onset Parkinson's disease; juvenile-onset Parkinson's disease; +, positive; -, negative; AD, autosomal dominant; AR, autosomal recessive; n/d, not determine; RF, risk factor.
(Belin and Westerlund, 2008; Chartier-Harlin et al., 2011; Cheon et al., 2012; Edvardson et al., 2012; Fujioka and Wszolek, 2012; Houlden and Singleton, 2012; Klein and Westenberger, 2012; Massano and Bhatia, 2012; Fujioka et al., 2013; Köroğlu et al., 2013; Bin-Umer et al., 2014; Olgiati et al., 2014; Tsika et al., 2014; Vilariño-Güell et al., 2014; Winkler et al., 2014; Wolters et al., 2014; Beilina and Cookson, 2015; Chen et al., 2015; Deng et al., 2015; De Rosa et al., 2015; Erro, 2015; Ruiz-Martinez et al., 2015)

## I. 5.2. Potential mechanisms of dopaminergic cell death

The causes of dopaminergic cell death occurring in PD remain unknown. However, different mechanisms have been proposed in order to explain the loss of those neurons in idiopathic and genetic PD. Protein misfolding and mitochondrial dysfunction leading to protein aggregation and oxidative stress respectively may constitute the molecular mechanisms at the basis of dopaminergic cell death. Inflammation is also a feature of PD neuropathology, and mounting evidence supports its contribution in neurodegeneration. These mechanisms have been found altered in PD and they could be interrelated, however there is no indication about the starting point.

### I. 5.2.1. Aggregation of misfolded proteins and dysfunction of the ubiquitin-proteasome/autophagy-lysosome systems

The abnormal deposition of proteinaceous materials in aggregate forms such as the Lewy bodies in PD, is a common feature of many age-related neurodegenerative diseases, including PD (Cook et al., 2012). In sporadic PD and more especially in PD patients with α-synuclein mutations, these aggregates from misfolded protein such as α-synuclein could be the initiation factor of the dopaminergic cell damage and death due to the impairment of intracellular trafficking, cell deformation and sequestration of proteins necessary for cell survival (Klein and Westenberger, 2012; Jankovic and Tolosa, 2015). This hypothesis is supported by the observation in aged brains of the high levels of oxidized proteins (e.g. α-synuclein), impairment of chaperone proteins involved in protein folding, ubiquitin-containing protein aggregates and the loss of functional proteasomal/autophagy-lysosomal system necessary for the protein degradation (Keller et al., 2002). All these impairments can induce a vicious cycle, where each damage or insult reinforces the proteins misfolding leading to cell death. Recently, a new hypothesis proposes for α-synuclein aggregation a prion-like spreading mechanism (Masuda-Suzukake et al., 2013; Chauhan and Jeans, 2015). This hypothesis suggests a neighbouring cell-to-cell spreading from abnormal α-synuclein structure to the "normal" α-synuclein, which leads to the aggregation of the "normal" α-synuclein and cell death.

### I. 5.2.2. Mitochondrial dysfunction and oxidative stress

Brain constitutes about 2% of total body but the brain mitochondrial consumption represents 20% of oxygen of the total body. Therefore, brain is highly exposed to the reactive oxygen species (ROS) metabolites produced by the mitochondrial respiratory chain (Uttara et al., 2009). Numerous studies in PD have reported a dysfunction of mitochondrial complex I with a higher level of ROS and a lower level of antioxidant glutathione in substantia nigra pars compacta (Ciccone et al., 2013). Inhibition of complex I induces an increase of oxidative metabolites, peroxynitrites and ROS leading to the damage of proteins, nucleic acids and lipids that in turn affect the mitochondrial activity and starts a cycle of ROS generation (Dias et al., 2013). In addition, the dopamine synthesis itself and the dopamine auto-oxidation occurring in dopaminergic neurons generates oxidative metabolites and ROS which are strongly linked to the damages of the neighbourhood proteins (see chapter: II. 5.1. Dopamine oxidation and oxidative stress in Parkinson's disease) (Meiser et al., 2013).

### I. 5.2.3. Inflammation

Anatomo-pathological studies have reported that brains from PD patients present a proinflammatory activation of the resident immune cells such as microglia and astrocyte (glial cells), but peripheral leukocytes are also involved in the inflammation process (Hunot and Hirsch, 2003; Whitton, 2007; Hirsch and Hunot, 2009; Hirsch et al., 2012). These leukocytes come from the blood vessel and infiltrate the brain though blood-brain barrier. These immune cells acquire phagocytic properties and the capacity to produce inflammatory mediators. In PD brain, various proinflammatory cytokines are detected in the CSF, in serum and in the affected brain regions such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), IL-2, IL-6 and interferon-γ (IFN-γ) (Boka et al., 1994; Bessler et al., 1999; Hunot et al., 1999; Rocha et al., 2015). Cytokines activate the production of nitric oxide and ROS through the increase of the expression of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, inducible nitric oxide synthase (iNOS) and cyclo-oxygenase 2 (COX2), which contributes to inflammation process (Boje and Arora, 1992; Hunot et al., 1996; Knott et al., 2000). The activation nuclear factor–κB (NF-κB) is detected in the substantia nigra in PD and is required for the transcription of proinflammatory molecules (Hunot et al., 1997). Cytokines are able to potentiate the activation of the glial cells and subsequently potentiate the inflammation (Jankovic and Tolosa, 2015). They also act directly on dopaminergic neurons by the activation of their receptive receptors. For instance, the activation of the TNF receptor

triggers an intracellular signalling cascade, which leads to cell death (Boka et al., 1994; Hunot et al., 1999). However, inflammation seems to be a secondary event in PD pathology and it is not a primary pathogenic event, which suggests a signal arising from injured neurons or from an alteration in the microenvironment (Jankovic and Tolosa, 2015). Based on this information, it is supposed that neuroinflammation does not initiate PD neurodegeneration but can amplify PD progression.

### I. 5.3. Environmental factors

The research of environmental factors potentially involved in PD has been significantly slower. Indeed, conducting clinical and experimental studies which investigate the role of environmental factors is more difficult for several reasons. Exposure to a toxic environment factor may occur before the onset of clinical manifestation and remains undetected (Di Monte et al., 2002; Goldman, 2014). Moreover, the complexity of these studies can also come from possible additive or synergistic effects of multiple exposures and from the interactions between environmental factors and genes (Di Monte et al., 2002). Despite these complexities, the studies on environmental factors may have far-reaching implications, including the development of preventive strategies and policies.

**Pesticides:** Organochloride insecticides (dichlorodiphenyltrichloroethane, DDT, for mosquito and dieldrin for termites) commercialized in 1940s and 1950s then banned in 1970s are the most commonly pesticides associated to PD. They double the risk to develop PD (Goldman, 2014). Organochlorides lead to the activation of ROS production and neuroinflammation as well as they induce the aggregation of α-synuclein and impairment of the ubiquitin-proteasome system (Goldman, 2014). Several other families of pesticides such as hexachlorohexanes, organophosphates (insecticide), 2,4-dichlorophenoxyacetic acid (herbicide), dithiocarbamates (fungicides) are also suggested to increase the risk for PD.

**Nicotine**: Despite the numerous adverse health effects of cigarette smoking, many studies have consistently found that smokers have a lower incidence of PD, which could related to the protective effect of nicotinic receptors activation (Thiriez et al., 2011; Quik et al., 2012).

**Coffee:** The consumption of coffee is also consistently associated to a reduction of the PD risk, probably due to the caffeine effect which is antagonist of adenosine A2A receptor (Kempster and Wahlqvist, 1994; Costa et al., 2010; Quik et al., 2012).

**Metal**: The iron plays a role in neurodegeneration process but it is still unclear whether it occurs prior the neurodegeneration or it is the consequence of the neurodegeneration (Dusek et al., 2015). High levels of redox-active irons could initiate the ROS production by the mitochondria, α-synuclein aggregation and neuroinflammation (Goldman, 2014; Dusek et al., 2015). The copper, lead, manganese, zinc and mercury are also suspected to be involved in PD (Stelmashook et al., 2014).

**Head injuries**: Demonstrated over the years, the traumatic brain injuries were associated with an increase in PD risk, probably by the involvement of inflammation, disruption of the blood-brain barrier, oxidative stress and genetic PD link with α-synuclein (Goldman et al., 2012; Surgucheva et al., 2014; Lee et al., 2015).

**Air pollution**: Currently, there is not clear evidence of the influence of air pollution on PD risk (Goldman, 2014). Nevertheless, recent data suggest that the long-term exposure to air pollution that can disrupt the blood-brain barrier and induce neuroinflammation and α-synuclein accumulation (Calderón-Garcidueñas et al., 2008).

## I. 5.4. Neurotoxins producing Parkinson disease's-like symptoms

### I. 5.4.1. MPTP

In 1982, several drug abusers in Northern California developed severe Parkinson-like symptoms because of an accidental intoxication from an even limited exposure of a contaminant in the synthetic heroin named 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983). This discovery gave the first indication that exogenous toxins may be involved in the PD pathophysiology (Langston et al., 1984). The clinical neurological features of MPTP intoxication are the same than observed in PD and respond to L-Dopa. Patients showed damage in the substantia nigra, but unlike PD, Lewy bodies were not present. MPTP is a lipophilic molecule that freely cross the blood-brain barrier (Riachi et al., 1989) and is readily metabolized by the glial monoamine oxidase type B (MAO-B) in toxic form, the 1-methyl-4-phenylpyridinium ($MPP^+$) (Rainbow et al., 1985). $MPP^+$ is

extruded via the organic cation transporter-3 (OCT3) into the extracellular compartments (Wu et al., 1998) and is taken up by the dopamine transporter (DAT) into dopaminergic neurons (Gessner et al., 1985; Javitch et al., 1985). The accumulation of $MPP^+$ inhibits the electron transport chain complex I of mitochondria (Ramsay and Singer, 1986; Klaidman et al., 1993), which induces ROS production and nigral toxic damages (Cohen, 1984). Currently, mice and monkeys treated with MPTP are reliable and widely used models for PD research (Tieu, 2011).

### I. 5.4.2. 6-OHDA

6-Hydroxydopamine (6-OHDA) was the first dopaminergic neurotoxin discovered and extensively used to generate PD models since 30 years (Ungerstedt, 1968; Schwarting and Huston, 1996; Tolwani et al., 1999). Like dopamine, 6-OHDA does not cross the blood-brain barrier that is why it is administered by a stereological injection into brain (Simola et al., 2007). 6-OHDA is a structural analogue of dopamine which can be taken up by DAT into dopaminergic neurons where it is oxidized, which leads to generate oxidative dopamine metabolites, free radicals and ROS and the subsequent cell damages and cell death (Simola et al., 2007).

### I. 5.4.3. Paraquat and rotenone

Paraquat (1,1'-dimethyl-4,4-bipyridinium) is a cationic herbicide widely used in agriculture fields during the $20^{th}$ century and discontinued in 2006 in France because its long-term exposure induces the development of PD (Goldman, 2014). This herbicide has structural similarities with $MPP^+$ and crosses the blood-brain barrier, probably through the neutral amino acid transporter (Corasaniti et al., 1991). Paraquat is also taken up by DAT into dopaminergic neurons (Rappold et al., 2011). Within neurons, paraquat produces hydroxyl free radicals and ROS leading to dopaminergic cell death.

Rotenone was originally extracted from Fabaceae plant and used by the indigenous people as a poison to catch fish (Goldman, 2014). Around the world, rotenone was commonly used as an organic unselective pesticide for vegetable gardens. Now, it is restricted to eradicate invasive fish species as a piscicide in lakes (Goldman, 2014). The advantage of rotenone is that it is easily degraded by exposure to sunlight and water, therefore, the likelihood of rotenone exposure is very low. Rotenone is very lipophilic and crosses easily

the blood-brain barrier to be distribute evenly throughout the brain (Cannon et al., 2009). Rotenone impairs mitochondrial function, reduces ATP production, generates ROS, and activates microglia (Cannon et al., 2009). Thus, these cellular dysfunctions induce the dopaminergic neuronal degeneration, which leads to mimics pathological hallmarks of PD including formation of Lewy bodies.

# I. 6. Parkinson's disease treatments

## I. 6.1. Pharmacologic treatments for restoration of dopamine depletion

The research on the PD pathophysiology and the development of PD animal models allowed the optimization of several therapeutic drugs. The first pharmacological approaches in PD consist to compensate the depletion of dopamine to alleviate motor dysfunction and to improve the patient's quality of life. Two strategies are used to rescue the dopamine depletion (Jankovic and Aguilar, 2008). Firstly, administration of a dopamine precursor or dopaminergic agonist receptors can compensate the loss dopamine. Secondly, the inhibitors of enzymes involved dopamine degradation can help to sustain the level of dopamine.

### I. 6.1.1. Levodopa (L-Dopa)

Discovered in 1960, L-Dopa is a precursor of the dopamine widely used in the management of PD symptoms (Carlsson et al., 1958; Birkmayer and Hornykiewicz, 1961; Cotzias et al., 1967, 1969). Unlike to the dopamine, L-Dopa crosses the blood-brain barrier and then it is converted into dopamine to restore the level of dopamine. The effectiveness of L-Dopa treatment on motor symptom in PD can be divided into three periods (Hauser, 2009; Nagatsua and Sawadab, 2009).

1. The first period of treatment corresponds to the 3 to 6 years with a dramatic beneficial improvement and an almost normal quality of life, described as the "honeymoon".
2. Then, this period is followed by a fluctuation on motor efficacy named wearing off. This second period corresponds to a loss of L-Dopa treatment effectiveness with the reappearance of motor symptoms. This effect is clearly associated to the treatment duration and is found in 90% of patients after 10 years of treatment. New symptoms are observed such as freezing and involuntary movements named dyskinesia. This

dyskinesia appears when the L-dopa plasma level is too high. Conversely, when the plasma level is low, akinesia and bradykinesia are present.

3. Finally, patients become non-responsive to L-Dopa. This is the most disabling period.

#### I. 6.1.2. Dopamine agonists

Dopamine agonists generate less syndrome effect and motor fluctuation than the L-Dopa, essentially due to the longer plasmatic elimination (Wolters et al., 2014). They are frequently used as monotherapy or in combination with L-Dopa to retard its dosage and the wearing-off symptoms (Clarke and Guttman, 2002). Developed in the 1980s, the first dopamine agonists are derivatives from ergot including bromocriptine, pergolide, carbergolin, apomorphine and lisuride (Horowski, 2007). However, their use is discouraged because of the important side-effects such as neuropsychiatric and cardiovascular disorders (Brooks, 2000). Only the apomorphine is still available in a self-injectable form essentially used as a rescue drug for people with severe freezing or off episodes (Wolters et al., 2014). Currently, the most widely used dopamine agonists are the pramipexole, ropinirole and rotigotine in prolonged formulation and in transdermal patch (Wolters et al., 2014).

#### I. 6.1.3. Inhibitors of dopamine degradation

Three therapeutic classes are used to block the degradation of dopamine or L-Dopa: the inhibitors of: 1) aromatic L-amino acid decarboxylase (DOPA decarboxylase, ADCC); 2) catechol-O-methyl transferase (COMT); 3) MAO-B.

ADCC inhibitors (DDCI), such as carbidopa and benzerzide, help to avoid the peripheral ADCC conversion of L-Dopa into dopamine (Pinder et al., 1976; Lieberman et al., 1984; Gershanik, 2015). These compounds do not cross the blood-brain barrier, which does not block the brain ADCC, essential for the brain conversion of L-Dopa into dopamine (Pinder et al., 1976; Lieberman et al., 1984; Gershanik, 2015).

COMT inhibitors, such as entacapone and tolcapone, block peripheral COMT metabolization of L-Dopa, which helps to increase its half-life up to 50% (Bonifácio et al., 2007; Leegwater-Kim and Waters, 2007; Marsala et al., 2012). Entacapone acts only peripherally whereas tolcapone also acts in the central nervous system, that is why tolcapone is probably more effective than entacapone (Bonifácio et al., 2007; Leegwater-Kim and

Waters, 2007; Marsala et al., 2012). However, due to the hepatoxicity side effect of tolcapone, it is mostly used as a second-line of COMT inhibitors.

The dopamine is also degraded by the monoamine oxidase B (MAO-B) (Marconi and Zwingers, 2014). Its inhibition by the selegiline and rasagiline helps to reduce the dose of L-dopa (Marconi and Zwingers, 2014).

### I. 6.2. Non-dopaminergic agents

The amantadine, an antagonist of N-methyl-D-aspartate-type (NMDA) glutamate receptor, is used in monotherapy or with L-Dopa to regulate the excess of glutamatergic activity and thus improves the motor symptoms of PD such as dyskinesia (Rajput et al., 1998; Connolly and Lang, 2014).

Anticholinergic drugs (trihexyphenidyl, biperiden, orphenadrine, procyclidine, ethopropazine, diphenhydramine) are used to reduce the hyperactivity induced by the striatal cholinergic tone (Katzenschlager et al., 2003). However, these drugs have important peripheral (dry mouth, blurred vision, constipation) and central nervous system (confusion and memory impairment) side effects.

The non-motor symptoms such as depression are treated with drugs commonly used in non-parkinsonian depression such as tricyclic antidepressants and serotoninergic and noradrenergic reuptake inhibitors (Paumier et al., 2012; Connolly and Lang, 2014).

### I. 6.3. Surgical treatment: deep brain stimulation

The deep brain stimulation of the subthalamic nucleus or globus pallidus internalis is a useful surgical procedure to improve locomotor symptoms in PD (Benazzouz et al., 1993; Pollak et al., 1993; Limousin et al., 1995). The aim of the stimulation is to changes brain activity in a controlled manner by the inhibition of the neuronal hyperactivity on the thalamus. Functionally, a pacemaker placed under the clavicle triggers the brain stimulation through two electrodes implanted bilaterally. This implantation improves the motor function that is preserved after 5 years and allows to stop or to reduce the L-Dopa treatment. Deep brain stimulation is rather reserved for patients refractory to pharmacologic therapy with

idiopathic PD and under 70 years of age (Volkmann, 2004; Perlmutter and Mink, 2006; Moldovan et al., 2015).

### I. 6.4. New strategies, targets and drugs to cure Parkinson's disease

Most of the current pharmacotherapeutic approaches in PD presented before aim to treat the symptomatic motor disorders by the principle of dopamine replenishment. Moreover these drugs provide symptomatic relief because many patients develop motor complications with long-term treatment (Mandel et al., 2003; Stayte and Vissel, 2014). Unfortunately, there is no available curative or disease-modifying treatment for neurodegenerative process so far (Jankovic and Aguilar, 2008). However, in last decade, many new strategies of treatment are currently being evaluated in preclinical research and for some have reached the clinical trials. Indeed, several new potential therapeutic targets have been proposed to restore motor functions, to reduce non-motors symptoms and drug side effects as well as for their neuroprotective and/or neurorestorative effects (listed in Table 6) (Mandel et al., 2003; Stayte and Vissel, 2014).

Great advance was also made in the field of gene therapy (Coune et al., 2012; Douglas, 2013; O'Connor and Boulis, 2015). It consists in an intraputaminal bilateral injection of a viral vector to overexpress an enzyme(s) involved in the synthesis of dopamine or GABA (listed in Table 6). These viral injections (ProSavin and AAV2-GAD) have already demonstrated an improvement in UPDRS scores in PD patients (LeWitt et al., 2011; Palfi et al., 2014).

The replacement of lost dopaminergic cell by neural transplantation was also already tested in human (listed in Table 6). For example, the transplantation of human embryonic stem cells was tried into the striatum of patients (Brundin et al., 2010; Freed et al., 2011; Barker et al., 2013). Despite good performance on motor symptoms, patients developed involuntary movement side effects, so called graft-induced dyskinesias. Additionally, an accumulation of α-synuclein was also observed, probably due to a contamination from the host tissue.

| Table 6. New strategies, targets and drugs to cure Parkinson's disease | |
|---|---|
| **Reduction of motor deficit and dyskinesia** | Antagonists of AMPA receptor: perampanel, talampanel, topiramate<br>Agonists of 5-HT1A, 5-HT1B receptor: sarizotan, buspirone, mirtazapine, eltoprazine<br>Antagonists of adrenergic receptor α2<br>Negative allosteric modulators of mGluR5 receptor: ADX48621-201<br>Antagonist of NMDA receptor and agonist of sigma 1: AVP-923 |
| **Neuroprotective / Neurorestorative therapies** | Antagonists of A2A receptor: istradefylline, preladenant, caffeine<br>Antagonists of mGluRI: dipraglurant, AFQ056<br>Calcium channel blockers (Cav1.3): isradipine<br>Agonist of GLP1R: exandin-4<br>Iron chelators<br>Anti-inflammatory drugs: PPAR-γ activator (pioglitazone)<br>Antioxidants: inosine, glutathione, coenzyme Q10, N-acetylcysteine<br>Neurotrophic factors: GDNF, neurturin, PYM50028 (cogane), CERE-120<br>ROCK inhibitors, statins, nicotinic agonists, GM1 ganglioside, NURR1 agonists, LXR agonists |
| **Strategies based on PD mutation** | Blockers of the α-synuclein accumulation<br>Increase the clearance of α-synuclein: phenylbutyrate<br>Active α-synuclein immunization: epitope based therapy PD01A, affitope and PD03A<br>Passive α-synuclein immunization: antibody based therapy, PRX002<br>Inhibitors of LRRK2<br>Other drugs related PD mutations |
| **Gene therapies - overexpression** | Combination of AADC, GCH1 and TH (Prosavin, lentiviral vector)<br>AAV2-GAD<br>Neurotrophic factors (AAV2-GDNF, AAV2-NTN: CERE-120) |
| **Dopaminergic neural grafting** | Fetal ventral mesencephalon grafting cell from abortion<br>Blastocyst-derived embryonic stem cell line differentiated into dopaminergic neurons (hESCs)<br>Somatic cells reprogrammed and differentiated into dopaminergic neurons (iPS cells) |

5HT, 5-hydroxytryptamine (serotonine); A2A, adenosine 2A; AADC, aromatic L-amino acid decarboxylase (catalyses L-Dopa in dopamine); AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; GAD, glutamate decarboxylase (catalyses the decarboxylation of glutamate into GABA); GCH1, GTP cyclohydrolase 1 (synthetizes the tetrahydrobiopterin, a co-factor of the tyrosine hydroxylase); GDNF, glial derived neurotrophic factor; GLP1R, glucagon-like peptide 1 receptor; hECSs, human embryonic stem cells; iPS, induced pluripotent stem cell; LXR, liver X receptors; mGluR, metabotropic glutamate receptor; NMDA, N-methyl-D-aspartate; NTN, neuturin; NURR1, nuclear receptor related 1 protein; PPAR, peroxisome proliferator-activated receptors; ROCK, rho-associated protein kinase ;
(Mandel et al., 2003; Brundin et al., 2010; Freed et al., 2011; Coune et al., 2012; Barker et al., 2013; Douglas, 2013; Stayte and Vissel, 2014; Kalia et al., 2015; O'Connor and Boulis, 2015) The Michael J. Fox Foundation for Parkinson's Research: https://www.michaeljfox.org/

# II. Dopaminergic synapse

Dopamine was synthesized for the first time by Barger and Ewens in 1910 (Fahn, 2008). In 1938, Holtz discovered the ADCC enzyme responsible to converting L-Dopa in dopamine (Holtz et al., 1938). The catecholaminergic pathway (L-Dopa, dopamine, norepinephrine and epinephrine) was proposed by end of the 1940s (Blaschko, 1939) and a decade later the link between the dopamine and brain function in the context of the pathophysiology of PD. Two studies identified the striatum as the area with the most important concentration of dopamine in the brain (Bertler and Rosengren, 1959; Sano et al., 1959).

## II. 1. Dopamine biosynthesis

The pathways for catecholamine synthesis are well known (Figure 4) (Elsworth and Roth, 1997; Daubner et al., 2011; Meiser et al., 2013). The amino acid L-tyrosine from the blood is the common precursor of all catecholamines. In synaptic terminal, the tyrosine hydroxylase (TH) catalyses the hydroxylation reaction of L-tyrosine to L-Dopa (Bayer and Pickel, 1990; Meiser et al., 2013). This rate-limiting step in the synthesis of dopamine (Nagatsu et al., 1964) is possible by the phosphorylated TH form which activity is dependent on calcium, cyclic adenosine monophosphate (cAMP) and tetrahydrobiopterin ($BH_4$) (Musacchio et al., 1971; Nakashima et al., 1971; Gutman and Segal, 1972; Nagatsu, 1995). At this point, the resulting molecule is L-Dopa, also used as a pharmacological treatment in PD. Then, DOPA decarboxylase enzyme synthetizes the L-Dopa in dopamine (Burkhard et al., 2001).

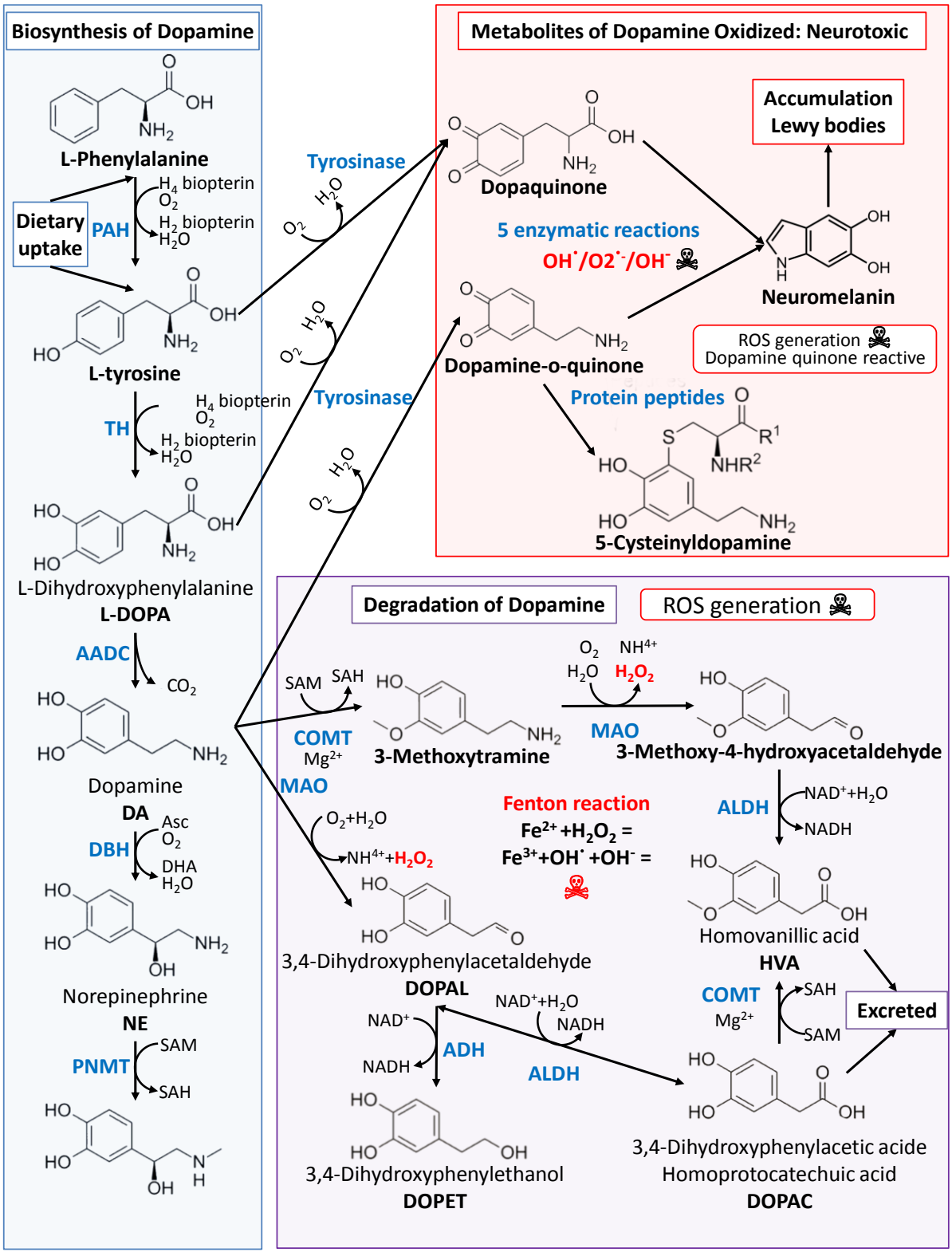


Figure 4. **Dopamine biosynthesis, degradation and oxidation products**

AADC, aromatic L-amino acid decarboxylase; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; Asc, ascorbate; COMT, catechol-O-methyl transferase; DBH, dopamine-β-hydroxylase; DHA, dehydroascorbic acid; MAO, monoamine oxidase; PAH, phenylalanine hydroxylase; PNMT, phenylethanolamine N-methyltransferase; ROS, reactive oxygen species; SAM, S-adenosyl-l-methionine; SAH, S-adenosyl-l-homocysteine; TH, tyrosine hydroxylase; $O2^{\cdot-}$, superoxide; $OH^{\cdot}$, hydroxyl radical; $OH^{-}$, hydroxide ion.

# II. 2. Dopaminergic synaptic vesicles and exo-endocytosis

The synaptic vesicle consists of spherical lipid bilayers with an external diameter of 40 nm (Figure 5) (Floor et al., 1988). Vesicles are formed in the Golgi apparatus, then, they are transported along microtubules using kinesin and dynein motors to go until presynaptic terminals (Oster et al., 1989). Plenty of proteins participate in the synaptic vesicles function (Morciano et al., 2005; Takamori et al., 2006; Burré and Volknandt, 2007; Volknandt and Karas, 2012). More than 80 proteins with transmembrane domains identified by proteomic studies are present in the vesicles (Boyken et al., 2013). Most of these vesicular proteins such as rabs, synaptotagmin and synaptobrevin are involved in the intracellular transport and exocytosis docking plasma membrane (Stein et al., 2003; Takamori et al., 2006; Schlager and Hoogenraad, 2009). Only a small fraction of proteins (approximately 10 copies per vesicles) plays the critical role to refill the vesicles with the neurotransmitters. Among these, we could find the vesicular glutamate transporter (VGluT), vesicular GABA transporter (VGAT), vesicular acetylcholine transporter (VAchT) and vesicular monoamine transporter (see chapter II. 4.3. Vesicular monoamine transporter) (Fei et al., 2008). The synthesized dopamine is packaged into the synaptic vesicles through the vesicular monoamine transporter (VMAT) (Nirenberg et al., 1996). Synaptic vesicles are also highly enriched in a putative transporter named synaptic vesicle glycoprotein 2 (SV2) (Floor and Feist, 1989; Takamori et al., 2006). Three isoforms have been characterized: SV2A, SV2B and SV2C (Bajjalieh et al., 1993; Janz et al., 1998). They belong to major facilitator superfamily (MFS) and possess 12 transmembrane domains highly glycosylated, but their substrates have not been clearly identified. SV2A and SV2B exhibit broad expression in the central nervous system (Bajjalieh et al., 1994). Besides its potential transport function, SV2A seems involved in the formation of exocytosis complex through the interaction with the synaptotagmins and thus it could be critical for release of vesicles (Janz et al., 1999; Crèvecœur et al., 2013). SV2C, the third member of the synaptic vesicles protein family, has a restricted distribution with a high level found in the basal ganglia, midbrain and brainstem, a pattern very similar to VMAT2-containing vesicles (Janz and Südhof, 1999). SV2C has been suggested to be a modulator of dopamine vesicle storage (Janz and Südhof, 1999; Dardou et al., 2011, 2013). Recently, two SV2C SNPs (single nucleotide polymorphism) have been identified to be protective factor against PD when they are associated to the cigarette-smoking (Hill-Burns et al., 2013). Two types of dopaminergic vesicles containing VMAT2 has been identified: the large dense core

vesicles (LDCVs) and the small synaptic vesicles (SSVs) with a diameter of 120-160 nm and 45-50 nm respectively (Liu et al., 1994; Erickson et al., 1996; Nirenberg et al., 1996, 1997a; Elsworth and Roth, 1997). The dopaminergic system of substantia nigra uses the SSVs for dopamine release and they are localized in axon terminals but also in the trans-Golgi network of neuronal perikarya, the tubulovesicles of smooth endoplasmic reticulum, and in the potential sites of vesicular membrane recycling (Nirenberg et al., 1995).

The release of synaptic vesicles is initiated by action potentials from the neuronal body (Neher and Sakaba, 2008) but only 10-20% of action potentials have an effect on exocytosis. Release of the vesicles takes place in specialized and structured areas in the presynaptic compartment named "active zone" which is a zone of the membrane associated to a macromolecular network and located on direct opposed side to the postsynaptic density (Harris et al., 1992; Spacek and Harris, 1998). A fraction of 6 to 8 vesicles is associated to the membrane in the active zone as a pool named "readily releasable pool" (Rizzoli and Betz, 2005; Denker and Rizzoli, 2010; Denker et al., 2011). Two other pools of vesicles are present in the terminal but not in the active zone: a reserve pool with 17 to 20 vesicles and a larger resting pool with more than 180 vesicles (Rizzoli and Betz, 2005; Denker and Rizzoli, 2010; Denker et al., 2011; Alabi and Tsien, 2012; Rizzoli, 2014). Both pools are essentially involved in the prolonged stimulation of action potentials which opens the $Ca^{2+}$ channels inducing an elevation level of $Ca^{2+}$ into the cytosol. This $Ca^{2+}$ increase is crucial for the fusion of the membrane vesicles and plasma membrane (Chen and Scheller, 2001; Jahn and Fasshauer, 2012). Indeed, many soluble N-ethylmaleimide-sensitive factor activating protein receptor of the vesicles (v-SNARE) and of the plasma membrane (target-SNARE, t-SNARE) such as synaptotagmin and syntaxin are calcium sensors. The $Ca^{2+}$ binding to SNARE proteins induces theirs conformational structural changes, which triggers the interaction between v-SNARE and t-SNARE. This interaction brings closer the vesicles to plasma membrane and allows their fusion, a mechanism named zipping (Jackson, 2010; Risselada and Grubmüller, 2012). Then, after the exocytosis, the vesicles are recycled back into the pool of vesicles (Harris and Weinberg, 2012; Rizzoli, 2014).

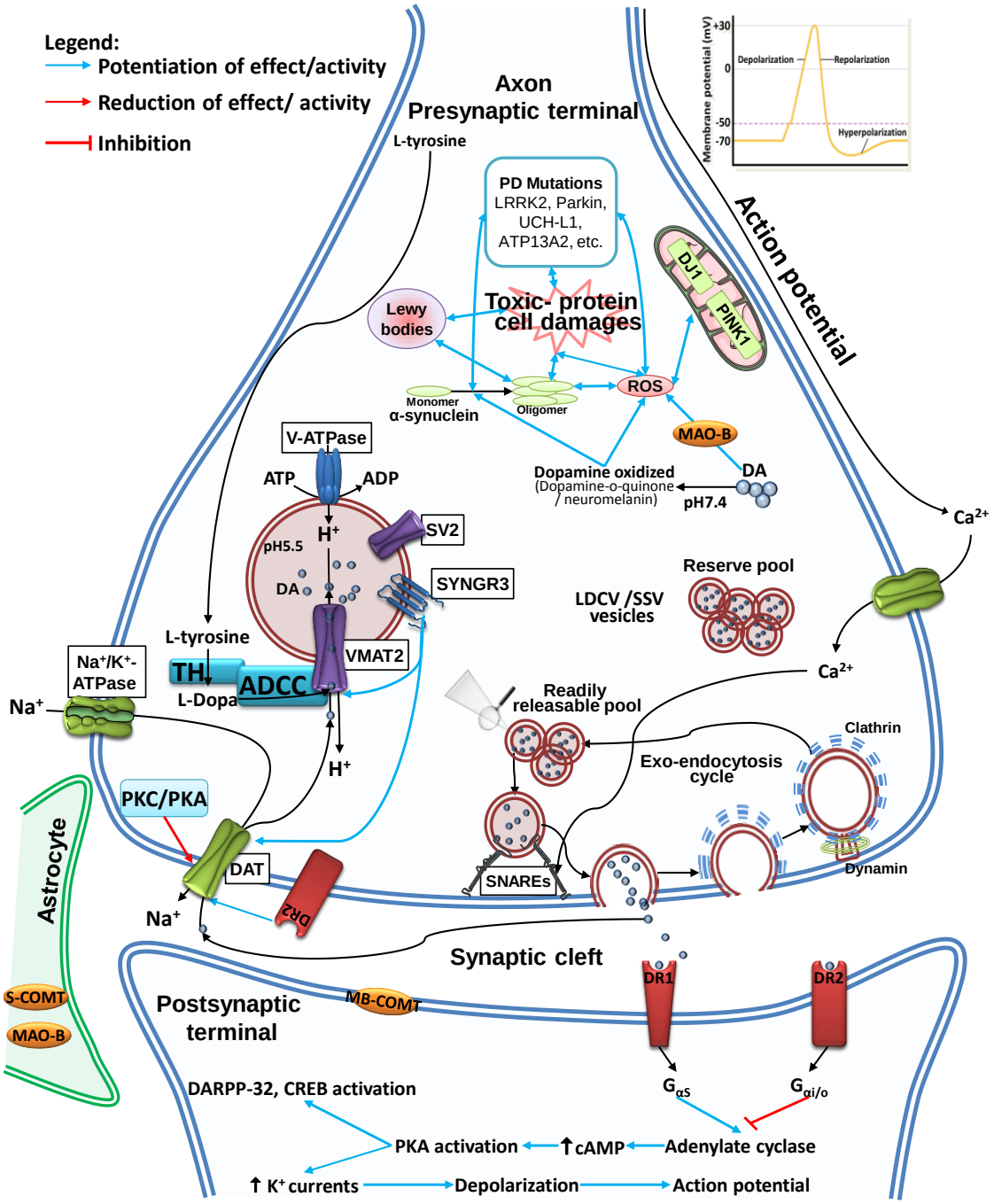


Figure 5. **Schematic of striatal dopaminergic synapse and mechanisms involved in Parkinson's disease neurodegeneration**

ADCC, aromatic L-amino acid decarboxylase; cAMP, cyclic adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CREB, cAMP response element-binding protein; DA, dopamine; DAT, dopamine transporter; DARPP-32, dopamine- and cAMP-regulated neuronal phosphoprotein; DR1, dopamine receptor D1; DR2, dopamine receptor D2; DJ1, protein deglycase; LDCV, large dense-core vesicle; LRRK2, leucine-rich repeat kinase 2; PINK1, PTEN-induced putative kinase 1; PKA, protein kinase A; PKC, protein kinase C; MAO, monoamine oxidase; MB-COMT, membrane-bound catechol-O-methyl transferase; ROS, reactive oxygen species; S-COMT, soluble catechol-O-methyl transferase; SSV, small synaptic vesicle;

VMAT2, vesicular monoamine transporter 2; SNARE, soluble N-ethylmaleimide-sensitive factor activating protein receptor; SV2, synaptic vesicle glycoprotein 2; SYNGR3, synaptogyrin 3; TH, tyrosine hydroxylase; UCHL-1, ubiquitin carboxy-terminal hydrolase L1; V-ATPase, vesicular ATPase;

# II. 3. Postsynaptic activation of dopamine receptors

Two families of dopamine receptors exist, D1 and D2. D1 family encompasses D1 and D5 receptors and D2 family encompasses D2, D3 and D4 receptors (Beaulieu et al., 2015). The most abundantly expressed receptor in the brain is the D1R and widespread in the caudate-putamen, nucleus accumbens, olfactory bulb, amygdala and frontal cortex (Cadet et al., 2010). D2R which is found in the caudate-putamen and nucleus accumbens is the second most abundantly expressed dopamine receptor (Meador-Woodruff and Mansour, 1991). D3, D4 and D5 receptors are less abundant and have restricted brain distribution.

All dopaminergic receptors are part to the large family G protein–coupled receptor (GPCR) (Sidhu and Niznik, 2000). GPCRs are composed of a central core domain consisting of seven-transmembrane domain for ligand binding and three subunits α, β and γ in heterotrimeric complex. Under the inactive state, α subunit is bound to the guanosine diphosphate (GDP). Once the ligand (dopamine) activates the GPCR, it leads to a conformational change of the receptor, which dissociates the GDP from the α subunit (Kobilka, 2007). Then, guanosine triphosphate (GTP) can take the empty place of the GDP, which dissociates the heterotrimeric G protein from the receptor and splits it into two active signalling elements: the α subunit and βγ complex. These subunits modulate various downstream specific effectors.

The classification of G proteins is based on the nature of the α subunit. D1R is coupled to $\alpha_s$ subunit which activates the adenylate cyclase whereas D2R is coupled to $\alpha_i$ which inhibits the adenylate cyclase (Figure 5) (Sidhu and Niznik, 2000). The adenylate cyclase produces the cAMP for the activation of the protein kinase A (PKA) which phosphorylates the potassium channels and DARPP-32 (dopamine- and cAMP-regulated neuronal phosphoprotein). Potassium channel phosphorylation reduces the potassium conductance and DARPP-32 phosphorylation can regulate neuronal electrical activity (Svenningsson et al., 2004). Both regulations induce a depolarization of the membrane which triggers the action potentials (Jaber et al., 1996; Missale et al., 1998; Beaulieu and Gainetdinov, 2011). Therefore, D1R activation provokes a stimulation of the postsynaptic neurons whereas D2R activation lowers the postsynaptic activity. Many other mechanisms

are modulated by dopamine receptor activation such as the calcium channel activity (Bigornia et al., 1990) and the transcriptional activity of the nuclear factor cAMP response element-binding protein (CREB) (Carlezon et al., 2005).

# II. 4. Dopamine recycling and transport into the synapse

## II. 4.1. Dopamine reuse and degradation

The reuse of dopamine requires a cycle of the reuptake dopamine in the presynaptic terminal through DAT followed by refilling of synaptic vesicles via VMAT2 (Figure 5) (Qi et al., 2008; Meiser et al., 2013; Schmitt et al., 2013). However, the dopamine can be degraded via a series of enzymes either by the dopaminergic neurons or by glial cells (Meiser et al., 2013). The neuronal cytosolic excess of dopamine is degraded by the MAO-B (Figure 4). MAO-B proceeds to oxidative deamination of dopamine into the 3,4-dihydroxyphenylacetaldehyde (DOPAL) and hydrogen peroxide (Edmondson et al., 2004). Then, alcohol dehydrogenase (ADH) or aldehyde dehydrogenase (ALDH) reduces or oxidizes DOPAL into 3,4-dihydroxyphenylethanol (DOPET) or 3,4-dihydroxyphenylacetic acid (DOPAC) respectively (Meiser et al., 2013)

The second pathway involved in the dopamine degradation is mediated through the glial cells using both MAO-B and soluble COMT (S-COMT) enzymes (Napolitano et al., 1995; Siderowf and Kurlan, 1999). S-COMT converts the DOPAC into homovanillic acid (HVA): the major metabolite of dopamine degradation. Finally, a membrane COMT (MB-COMT) anchored in the postsynaptic membrane can degrade the dopamine directly into the synaptic cleft (Roth, 1992; Myöhänen et al., 2010; Chen et al., 2011a). Both DOPAC and HVA reach the systemic circulation to be excreted in urine (Siirtola et al., 1975).

## II. 4.2. Dopamine transporter (DAT)

### II. 4.2.1. Structure and function of DAT

The amount of dopamine dictates the endurance of synaptic transmission and it seems critical to regulate the level of dopamine in the synaptic cleft. To this end, DAT regulates the level of dopamine by the reuptake of dopamine from the synaptic cleft to the synaptic terminal cytosol (Uhl, 2003; Leviel, 2011; Schmitt et al., 2013). The DAT function is crucial in terminal dopaminergic neurons because it provides 95% of dopamine of the intracellular stores (Jones et al., 1998). DAT is mainly localized in the plasma membrane close to the exocytosis site (active zones) of dopaminergic vesicles in the perisynaptic areas (Nirenberg et al., 1997a, 1997b). DAT mRNA is located in the dopaminergic cell bodies and nerve terminals including the basal ganglia and substantia nigra pars compacta (Ciliax et al., 1995; Freed et al., 1995; Haber, 2014).

DAT (69 kDa for human) forms a dimer or a tretramer (Hastrup et al., 2001, 2003) and contains 12 transmembrane domains, an intracellular C- and N-terminals and glycosylation sites at the N-terminus and within the intracellular loop between transmembrane domains 3 and 4 (Patel et al., 1993; Vaughan et al., 1996). It is a $Na^+/CL^-$ dependent transporter belonging to the family of solute carrier 6 (SLC6A3), closely related to the two other monoamine transporters: the norepinephrine transporter (NET, SLC6A2) and the serotonin transporter (SERT, SLC6A4) (Chen and Reith, 2000; Vallone et al., 2000; Kristensen et al., 2011). DAT uses the transmembrane $Na^+$ gradient between the extracellular space and the cytosol as a driving force to transport dopamine across the plasma membrane. Two $Na^+$ and one $Cl^-$ ions are co-transported with one dopamine molecule. Thus, dopamine transport depends on the gradient essentially established by the ubiquitous plasma membrane $Na^+/K^+$-ATPase.

### II. 4.2.2. DAT regulation

DAT is highly regulated in the plasma membrane and subsequently it modulates the dopaminergic signalling due to either attenuation or intensification of the dopamine reuptake (Mortensen and Amara, 2003; Torres et al., 2003b; Torres, 2006; Eriksen et al., 2010). It has been shown that the activation of PKC with phorbol 12-myristate 13-acetate (PMA) downregulates the DAT transport activity (Copeland et al., 1996; Vaughan et al., 1997; Zhu et al., 1997; Daniels and Amara, 1999; Melikian and Buckley, 1999; Chi and Reith, 2003;

Sorkina et al., 2005). Indeed, PKC activation induces the internalization of DAT through a rapid clathrin-dependent endocytosis process of the plasma membrane and then DAT is either degraded or recycled back to the plasma membrane (Sorkina et al., 2005). The molecular mechanism involved in the PKC-induced DAT internalization remains unclear. It seems to be related to the phosphorylation activity of PKC on DAT but it is also probably due to an activity of ubiquitination on the N-terminus domain of DAT. Studies using DAT-specific mutations of lysine positions (Lys19, Lys27, Lys35) demonstrated a clear absence of DAT ubiquitination and of PMA-induced DAT internalization (Miranda et al., 2007). Several other partners have been described in the mechanism of internalization such as phosphoinositide 3-kinase (PI3K)/Akt, protein kinase A (PKA) and neural precursor cell expressed developmentally downregulated gene 4-like (NEDD-4-2) (Sorkina et al., 2006). It has been shown that inhibition of Akt or the expression of a dominant negative mutant of Akt reduces the DAT cell surface in human embryonic kidney 293 (HEK293) transfected cell with DAT (Carvelli et al., 2002; Lin et al., 2003; Garcia et al., 2005).

The synaptic vesicles could regulate the DAT uptake by several SNARE proteins such as the synthaxin 1A and the synaptogyrin 3 (SYNGR3). DAT interacts with syntaxin 1A in the plasma membrane which in turn interacts with the vesicular synaptotagmin 1 (Lee et al., 2004; Binda et al., 2008; Carvelli et al., 2008). The overexpression of syntaxin 1A in LLCPK1 cells reduces the maximal rate velocity ($V_{max}$) of dopamine uptake, probably due to DAT internalization, but, which is independent to PKC activity (Cervinski et al., 2010). The synaptic vesicle protein SYNGR3, it is a specific protein of dopaminergic systems expressed in the basal ganglia and substantia nigra (Belizaire et al., 2004) which seems to regulate positively the dopamine uptake of DAT (Egana et al., 2009). The co-immunoprecipitation studies using striatal mouse tissue and co-transfected cells with SYNGR3 and DAT showed an interaction between the N-terminus of DAT and the cytoplasmic N-terminus of SYNGR3. SYNGR3 overexpression increases the DAT activity whereas SYNGR3 downregulation with siRNA reduces the DAT activity. These data suggest a possible interaction mechanism between DAT and the vesicles in order to have an efficient dopamine uptake through both DAT and VMAT2 directly into the synaptic vesicles what may avoid an increase in cytosolic dopamine (Egana et al., 2009). Several *in-vitro* and *in-vivo* studies have demonstrated a positive effect on the dopamine uptake through an increase of the DAT surface expression induced by D2R expression (Lee et al., 2009).

### II. 4.2.3. DAT inhibitors

DAT is the target of several psychostimulants such as cocaine and amphetamine. Potent and selective compounds inhibiting DAT have been identified. Among others, WIN 35,428, 3-CPMT and GBR-12935 are the most potent compounds blocking the reuptake of dopamine and subsequently increasing the extracellular concentration of dopamine (Kintscher, 2012; Huot et al., 2015). Nevertheless, they display an activity also in other neurotransmitter transporters such as NET and SERT. Several numerous radiolabeled DAT inhibitors (WIN 35,428, RTI-121 or 55) are available for positron emission tomography (PET)- or single-photon emission computed tomography (SPECT)-ligands allowing to determine DAT binding in nigro-striatal system and thus to evaluate PD neurodegenerative progression (Marshall and Grosset, 2003; Brooks, 2004).

## II. 4.3. Vesicular monoamine transporter 2 (VMAT2)

### II. 4.3.1. Structure and function of VMAT2

VMATs are responsible for the efficient reuptake and storage of monoamines from the cytosol to the synaptic vesicles, which is crucial for proper monoaminergic neurotransmission in dopaminergic system (Carlsson et al., 1962; Kirshner, 1962; Wimalasena, 2011). VMATs contain 12 transmembrane domains and a cytosolic C- and N-terminals (Wimalasena, 2011). The intravesicular loop between 1 and 2 transmembrane domains contains three or four glycosylated sites (Yao and Hersh, 2007; Cruz-Muros et al., 2008). Indeed, VMATs are acidic glycoproteins with an apparent higher molecular weight of 70 kDa, but the native form of VMATs is also present with a relative molecular weight of 55 kDa. In addition, immunoblot detection also revealed a third VMATs band at low molecular weight (40 kDa) which correspond to a truncated form (Jassen et al., 2005; Tong et al., 2011). Yet, its function remains unclear.

Two VMAT isoforms (VMAT1 and VMAT2) with high homology have been identified (Erickson et al., 1996). VMATs belong to SLC18 transporter family and the MFS. VMAT2 (SLC18A2) is expressed in the central nervous system expression and in the endocrine and neuroendocrine system (Weihe et al., 1994; Peter et al., 1995; Schütz et al., 1998). Not only dopamine, but also norepinephrine, epinephrine, histamine, and serotonin can be transported by VMAT2. VMAT1 (SLC18A1) is exclusively expressed in

neuroendocrine system including chromaffin and enterochromaffin cells and it transports the same neurotransmitters as VMAT2 except histamine.

The VMAT-mediated dopamine transport into vesicles is powered by a transmembrane electrochemical $H^+$ gradient. This gradient helps to counteract the high concentration gradient of dopamine between the outside and the inside of the vesicles (Knoth et al., 1981; Nguyen et al., 1998; Beyenbach and Wieczorek, 2006). Vacuolar-type $H^+$-ATPase (V-ATPase) generates the $H^+$ gradient, which maintains a low intravesicular pH of 5.5. VMAT2 effluxes two protons out from the vesicle and reversely transports one molecule of dopamine as an antiport (Phillips and Apps, 1980; Eiden et al., 2004). First, one proton is extruded from the vesicle in order to generate a high-affinity binding site for dopamine on the cytosolic face. Then, VMAT ejects a second proton, which induces a VMAT2 conformational change for the incorporation of the dopamine molecule into the vesicle.

VMAT2 presents a low $K_m$ (affinity of the transporter for the substrate) in the micromolar range compared to other transporter such as VGluT, VGaT and VAchT (millimolar range) (Liu and Edwards, 1997; Blakely and Edwards, 2012). This low $K_m$ indicates a robust efficacy of VMAT2 in the dopamine uptake, which helps to reduce dopamine accumulation into the cytosol. It is important to note that the dopamine contained into the synaptic vesicles is concentrated to approximately at 100 µM, which is 10–1000 times higher than the cytosolic level (Elsworth and Roth, 1997).

### II. 4.3.2. VMAT2 regulation

The VMAT2 regulation remains poorly understood. It has been shown that $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and PKA phosphorylate the acid patch motif at the extreme C-terminus of VMAT2 without modifying dopamine uptake (Krantz et al., 1997; Yao et al., 2004; Chen et al., 2011b). It has been suggested that the phosphorylation could play a role in VMAT2 trafficking and subsequently influences its subcellular localization (Waites et al., 2001). Moreover, several studies have reported that the heterotrimeric G protein $G_{\alpha o2}$ and $G_{\alpha q}$ are able to downregulate the expression of VMATs in PC12 cells and in primary cultures, which leads to the monoamine uptake reduction (Ahnert-Hilger et al., 1998; Höltje et al., 2003; Winter et al., 2005; Brunk et al., 2006). A recent report suggests a coupled interaction complex with TH and ADCC enzymes and VMAT2, which helps to avoid the accumulation of dopamine into the cytosol (Cartier et al., 2010). D2R

activation with quinpirol agonist showed an upregulation of the vesicular dopamine uptake in purified striatal vesicles (Truong et al., 2004). This effect has been proposed as subsequent redistribution of VMAT2 in the membrane protein of the cytoplasmic non-membrane associated vesicles towards the plasmalemmal membrane-associated vesicles (Fleckenstein et al., 2007).

### II. 4.3.3. VMAT2 inhibitors

Reserpine and tetrabenazine are the most important identified inhibitors of VMAT2 (Rudnick et al., 1990; Schuldiner et al., 1993; Erickson and Varoqui, 2000; Chaudhry et al., 2008; Wimalasena, 2011). Amphetamine-like psychostimulants are also known to bind VMAT2 but they promote the release of monoamine vesicular stores (Floor and Meng, 1996).

Reserpine, an alkaloid historically used to treat high blood pressure (Slim et al., 2011), is a high-affinity competitive inhibitor of VMAT2 blocking irreversibly the transport (Parti et al., 1987) and the quantal release of dopamine (Colliver et al., 2000). The binding site of reserpine is the same as dopamine, which prevents the conformational change of VMAT2, the efflux of the second proton and the return to initial state (Zallakian et al., 1982). This binding mechanism explains the modulation of the transmembrane pH gradient and the irreversibility component of reserpine (Zallakian et al., 1982). The reserpine-treated rodent was an acute transitory PD models employed in research (Roos and Steg, 1964; Duty and Jenner, 2011). Reserpine induces a loss of storage capacity and a depletion of monoamine in the brain, which reproduces some symptoms associated to PD. However, reserpine-treated rodents are not accurate models due to the lack of selectivity for the inhibition of intravesicular dopamine reuptake, it also depletes the storage of the noradrenaline and serotonin.

TBZ is a high-affinity non-competitive inhibitor of VMAT2 with short action duration. Although not yet identified, the TBZ-sensitive binding site seems to be different from the reserpine-sensitive one (Ugolev et al., 2013). Indeed, studies reported a binding site independent of the pH gradient (Scherman and Henry, 1982) and non-inhibited by the reserpine or by the high concentration of dopamine (>100 fold to TBZ affinity binding) (Ugolev et al., 2013). Derivative radiolabeled dihydrotetrabenazine is used in image studies for measuring its binding level on VMAT2 for the assessment of PD neurodegeneration

(Martin et al., 2008). Moreover, TBZ is used for treatment of abnormal hyperkinetic involuntary movements in Huntington's chorea (Ondo et al., 2002)

# II. 5. Dopamine toxicity and transport dysregulation in Parkinson's disease

Compelling evidence suggests that degeneration of dopaminergic neurons in PD begins at synaptic terminals and axons (Morales et al., 2015). At the onset of motor disturbance in PD, 70% of dopamine and 50% of proteins involved in metabolism of dopamine such as TH, DAT and VMAT2 are lost in the striatum, whereas only 50% of dopaminergic neurons in substantia nigra is lost at this time. This observation suggest an initiation of pathogenesis in the synapse of nigral neurons (Coleman, 2005; Stephenson et al., 2007; Cheng et al., 2010; Tian et al., 2012).

## II. 5.1. Dopamine oxidation and oxidative stress in Parkinson's disease

The dopamine catechol ring is unstable under the neutral cell physiological conditions (pH 7.4), which results in a spontaneous oxidation due to the dissociation of the proton to the oxygen in the hydroxyl groups (Cabbat et al., 1985; Pham et al., 2009). This oxidation process produces the many toxic metabolites such as dopamine-o-quinone, cysteinyl-catechol, neuromelanin (ultimate product of dopamine oxidation) and ROS such as hydrogen peroxide and superoxide) (Meiser et al., 2013). Additionally, the dopamine degradation via the MAO-B enzyme produces hydrogen peroxide which is converted by Fenton reaction to the hydroxyl radicals in presence of ferrous iron (Youdim and Bakhle, 2006).

Several studies demonstrated that the accumulation of these dopamine metabolites results in a neurotoxicity and oxidative stress in PD (Jenner and Olanow, 1996). The post-mortem studies on PD brain patients showed the presence of the high level of oxidized proteins, lipids, and nucleic acids (Kumar et al., 2012). The neuromelanin (Spencer et al., 1998; Zecca et al., 2001, 2003) and cysteinyl-catechol were also found in the nigral region of PD brains (Miyazaki and Asanuma, 2008). Moreover despite a lake of neurotoxic effect of dopamine in one study (Santiago et al., 2000), typically the stereotaxic injection of dopamine in rat striatum provokes a dopaminergic cells loss which can be rescued by the antioxidant agents (Hastings et al., 1996a, 1996b; Rabinovic et al., 2000). The neurotoxic effects of

dopamine were confirmed in the midbrain neuron cultures and neuronal-like cells (Graham et al., 1978; Masserano et al., 1996; Koshimura et al., 2000; Zhang et al., 2000). Dopamine metabolites impair several crucial mechanisms and structures of the cells such as mitochondria, lysosome and proteasome system (Asanuma et al., 2004; Muñoz et al., 2012 p.20012; Dias et al., 2013; Segura-Aguilar et al., 2014; Blesa et al., 2015). Additionally, oxidative dopamine induces the α-synuclein misfolding and its aggregation (Maguire-Zeiss et al., 2005; Mosharov et al., 2009; Chan et al., 2012). All these data strengthen the evidence of the involvement of the dopamine oxidative stress in dopaminergic cell death.

### II. 5.2. DAT in Parkinson's disease

In agreement with the degeneration observed in the substantia nigra, imaging studies using radiolabeled markers have shown a decrease of DAT expression in PD in correlation to the stage disease severity (Bannon and Whitty, 1997; Nutt et al., 2004; Bannon, 2005; González-Hernández et al., 2010; Vaughan and Foster, 2013; Politis, 2014). In human PD, the loss of dopaminergic neurons correlates with the level of DAT expression. More the level of DAT is high, more dopaminergic neurons are vulnerable and match with a robust preferential neuron degeneration especially in the midbrain observed with topographic pattern of DAT protein expression (Cerruti et al., 1993; Uhl et al., 1994; González-Hernández et al., 2004). The glycosylated DAT is predominant in terminals of nigrostriatal neurons compared to other dopaminergic neurons (e.g. mesolimbic) and displays a higher dopamine uptake (Torres et al., 2003a; Li et al., 2004). The authors suggest that glycosylation level can explain the higher dopamine uptake and vulnerability of nigrostriatal neurons in PD (Afonso-Oramas et al., 2009). Regarding DAT mutation, only one DAT SNP has been detected in infantile patients with severe parkinsonism-dystonia, probably due to the DAT inactivation (Blackstone, 2009; Kurian et al., 2011).

Animal and in *in-vitro* studies have shown that DAT overexpression increases neurodegeneration. High sensitivity of dopaminergic cells were observed after treatment with neurotoxic agents (e.g. $MPP^+$, MPTP, paraquat and rotenone) in DAT ectopic expression (Donovan et al., 1999; Rappold et al., 2011; Masoud et al., 2015). These data have been confirmed in *in-vitro* neuroblastoma SK-N-MC cells and non-neuronal HEK293 and COS cells (Pifl et al., 1993; Kitayama et al., 1998; Storch et al., 1999, 2002). In contrast, DAT knockout animals and treatment with DAT inhibitors led to a higher resistance to neurotoxic

agents (Gainetdinov et al., 1997; Martins et al., 2013). Therefore, DAT seems the principal gateway for neurotoxicity and its dysregulation may contribute to disease initiation and/or progression (Bougria et al., 1995; Miller et al., 1999; Kurosaki et al., 2003; Hirata et al., 2008; Vaughan and Foster, 2013).

α-synuclein and parkin have been shown to impact DAT, suggesting a role in PD (Sidhu et al., 2004a). α-synuclein binds to CaMKII site at C-terminus of DAT and stimulates the ion current mediated by the DAT (Swant et al., 2011), which has been confirmed by the formation of a DAT and α-synuclein heteromeric complex in the cells co-transfected and in the rat mesencephalic neurons (Lee et al., 2001; Wersinger and Sidhu, 2003; Wersinger et al., 2003). It has been shown that the co-expression of α-synuclein and DAT in Lkt-mouse fibroblasts decreases the dopamine uptake ($V_{max}$ reduction of 30-50%) compared to DAT transfection alone (Wersinger and Sidhu, 2003; Wersinger et al., 2003). α-synuclein downregulation with siRNA induced a reduction of DAT $V_{max}$ dopamine uptake and of DAT inhibitor binding (WIN35-428) (Fountaine and Wade-Martins, 2007). Moreover, α-synuclein mutation A30P and A53T increase the sensitivity of HEK293-DAT transfected cells to the neurotoxins (6-OHDA, $MPP^+$ and rotenone) (Lehmensiek et al., 2002, 2006).

The E3 ubiquitin ligase (parkin) also regulates the DAT by its ubiquitination and degradation. Parkin induces a stabilization of the DAT at the cell surface which is associated to an increase dopamine uptake (Jiang et al., 2004). However, this result is inconsistent with the data which show that parkin disrupts the interaction between DAT and α-synuclein leading to a decrease of dopamine uptake neurotoxicity (Moszczynska et al., 2007).

Several studies have been reported the therapeutic potential of DAT inhibitors in PD. The increase of the extracellular dopamine for the potentiation of postsynaptic activity and the blockage of the entry of neurotoxic agents in the presynaptic terminal induced by the DAT inhibitors could be beneficial in PD (Nutt et al., 2004; Huot et al., 2015). Data in rodents and non-human primates PD models suggest that DAT inhibitors such as modafinil, amineptine, methylphenidate, SEP-228,791 or vanorexine may have neuroprotective effects against MPTP intoxication, although they have demonstrated no clear effectiveness on motor symptoms in the clinical trials in PD (Nutt et al., 2004; Huot et al., 2015).

## II. 5.3. VMAT2 in Parkinson's disease

Numerous studies suggest the potential role of VMAT2 function in human Parkinsonism (Liu and Edwards, 1997; Lotharius and Brundin, 2002; Alter et al., 2013; Lohr and Miller, 2014). Post-mortem PD brains display a severe reduction of VMAT2 greater than what could be explained by dopaminergic terminal loss (Pifl et al., 2014). In recent study in Italy, two SNP polymorphisms within the VMAT2 gene promoter are inversely associated to PD, presumably due to the increase in VMAT2 expression leading to a higher vesicular dopamine reuptake (Brighina et al., 2013). A lower incidence of PD in women was also found due to SNP gain-of-function in the VMAT2 promoter, thus conferring a neuroprotective effect selective for females (Glatt et al., 2006; Lin et al., 2010b). In contrast, a SNP in the coding exon of VMAT2 was identified in a Saudi Arabian family causing an infantile parkinsonian with profound motor and cognitive impairments, probably due to a reduction of monoamine transport (Rilstone et al., 2013). VMAT2 polymorphism and its expression level could also be associated to a variety of neuropsychiatric illness linked to a dopamine disorder such as schizophrenia, depression, Tourette's syndrome and bipolar disorder (Lajtha and Mikoshiba, 2009).

In *in-vivo* models, VMAT2 knockdown in mice induces a high oxidative stress, α-synuclein accumulation and progressive degeneration of the nigrostriatal neurons as well as a reduction of dopamine storage, transport and quantal release (Fon et al., 1997; Takahashi et al., 1997; Wang et al., 1997; Gainetdinov et al., 1998; Miller et al., 2001; Caudle et al., 2007; Taylor et al., 2011; Lohr et al., 2015). These mice display a high sensitivity of dopaminergic neurons to neurotoxic agents whereas mice overexpressing VMAT2 are less sensitive to neurotoxic MPTP insult (Lohr et al., 2014). In agreement, cultured midbrain neurons and cell lines overexpressing VMAT2 showed a protective effect against neuronal damage associated to an increase of storage capacity and dopamine release (Chen et al., 2005a; Vergo et al., 2007; Muñoz et al., 2012; Choi et al., 2015b). Interestingly, it has been suggested that increased VMAT2 $V_{max}$ uptake of dopamine and $MPP^+$ (2-fold elevation) observed in rat dopaminergic neurons may confer the resistance to MPTP-induced neurotoxicity compared to other species such as mice, primate and human (Staal and Sonsalla, 2000; Staal et al., 2000). VMAT2 inhibition induced by Ro 4-1284 and reserpine enhances the $MPP^+$ or MPTP neurotoxicity by a reduced sequestration into the vesicles (Staal and Sonsalla, 2000). It was

demonstrated that VMAT2 reduction in MPTP primate PD model is the key pathogenic event that precedes nigro-striatal dopaminergic neurons loss(Chen et al., 2008).

Strong evidence shows a direct link between α-synuclein and VMAT2. α-synuclein protofibrils may permeabilize the vesicles by its binding to VMAT2. This interaction induces the formation of an annular pore-like in the vesicular membrane, which may lead to a leakage of monoamines into the cytosol (Lashuel et al., 2002; Lotharius and Brundin, 2002; Volles and Lansbury, 2002, 2003; Zhu et al., 2003; Sidhu et al., 2004b; Guo et al., 2008). Moreover, *in-vitro* overexpression of α-synuclein in the SH-SY5Y cells causes a downregulation of the expression of VMAT2 proteins which is associated to a decrease of vesicular dopamine uptake and an increase in cytosolic dopamine and ROS (Mosharov et al., 2006; Guo et al., 2008).

### II. 5.4. VMAT2, a potential therapeutic target

All these data support the interest of VMAT2 as a potential therapeutic target for PD through the prevention of dopaminergic cytosolic oxidative stress by several mechanisms (Figure 6) (Zheng et al., 2006; Bernstein et al., 2014; Lohr and Miller, 2014; Yulug et al., 2015):

1. Reduction of dopamine oxidation into cytosol through the uptake of dopamine into the vesicles.

2. Blockage of dopamine oxidation into vesicles due to the low intravesicular pH (strong bond of the dopamine proton to the oxygen in the hydroxyl groups).

3. Sequestration of neurotoxic agents into the vesicles.

Today no VMAT2 activator has been identified, however some studies have proposed potential neuroprotective effects related to VMAT2 activation. Methylphenidate, a DAT inhibitor, is widely used in attention deficit hyperactivity disorder (ADHD) (Sandoval et al., 2002; Volz, 2008; Volz et al., 2009; Nandhagopal et al., 2011). Additionally to increase the brain dopamine level, methylphenidate also increases dopamine storage into synaptic vesicles and dopamine release from vesicles after potassium stimulation. It was suggested that the effects could be due to an increase in VMAT2 expression level. Subsequently, methylphenidate could improve the motor deficits in PD. Similarly to methylphenidate, pramipexole, which is a D2/D3 receptor agonist used in treatment of PD, is associated to a potential neuroprotective effect involving VMAT2 (Zou et al., 1999). Indeed, synaptic

vesicles from rat striatum treated pramipexole display a higher VMAT2 dopamine uptake activity. In *In-vitro* experiments, pramipexole treatment of the SH-SY5Y cells showed a time and dose-dependent increase in mRNAs and proteins of nuclear receptor related 1 protein (NURR1), DAT and VMAT2 (Pan et al., 2005).

However, the regulation of dopamine in neuronal terminal is a delicate balance. The aim is to keep the dopamine at the right place and time. When there is too much dopamine outside the vesicle, the dopamine becomes toxic. Reciprocally, too much dopamine packaged induses a less effective postsynaptic stimulation. And to make things even more complicated, a recent hypothesis suggests an interaction between DAT and VMAT2 leading to a mutual modulation of the dopamine reuptake, a fairly logical idea in order to have a fine regulation of dopamine into the cells (Egana et al., 2009).

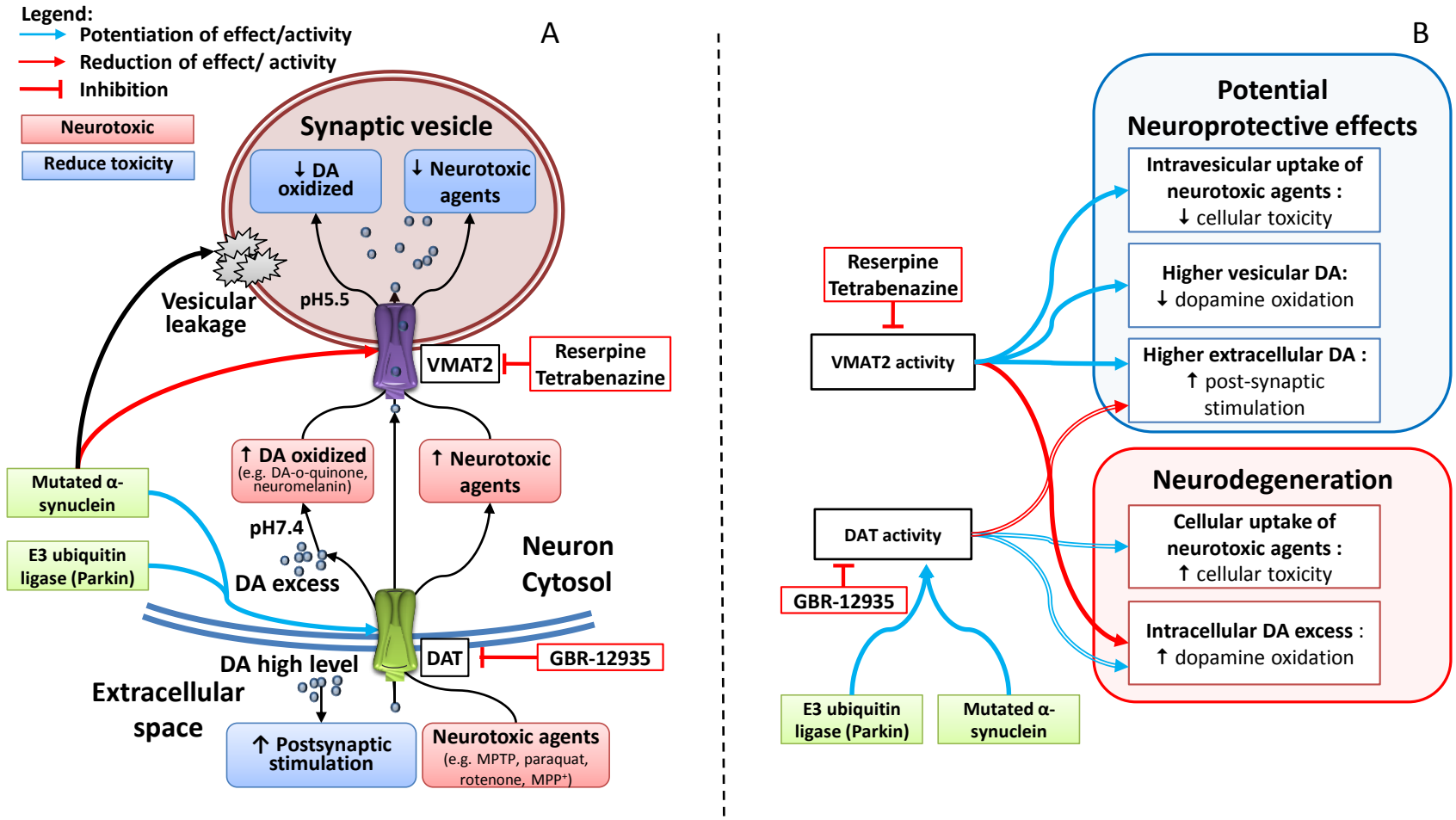


Figure 6. **Hypothetical neuroprotective and neurodegenerative mechanisms of dopamine transporter and vesicular monoamine transporter 2 involved in Parkinson's disease**

A. Cartoon of hypothetical transport mechanisms involved in the detoxification of oxidized dopamine and neurotoxic agents. This cartoon present also the neuroprotective and neurodegenerative components in Parkinson's disease

B. Schematic diagram of hypothetical neuroprotective and neurodegenerative mechanisms involving the activities of the dopamine transporter and vesicular monoamine transporter 2.

DA, dopamine; DAT, dopamine transporter; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; VMAT2, vesicular monoamine transporter 2;

# II. 6. Dopaminegic neurodegeneration in Parkinson's disease and the therapeutic potential of dopaminergic differentiation, neurogenesis and neurorestoration

Current therapies of PD target motor symptoms and do not cure, prevent or slow down the progression of dopaminergic cell loss. In this context, the neurorestoration and neuroregenerative therapies aim to fill a huge unmet medical need and represent a major challenge for human societies. Although some PD symptoms may appear before the complete dopaminergic cell loss, at the time of diagnosis about 70% decrease of dopamine level and about 50% of dopaminergic neurons are lost (Bernheimer et al., 1973; Kish et al., 1988; McGeer et al., 1988). Moreover, axonal projections to the striatum from the substantia nigra disappear by retrograde degeneration called dying-back phenomenon (Jellinger, 1999; Cheng et al., 2010). These observations suggest the possibility of a therapeutic intervention for reversing this degeneration.

## II. 6.1. Axonal and dendritic degeneration in Parkinson's disease

Several mechanisms were described in the dysregulation and degeneration of dopaminergic axons and dentrites in PD. The main factors triggering above mechanisms are neurotoxic agents (Spencer, 2000; O'Malley, 2010) as well as α-synuclein (Waxman and Giasson, 2009; Bellucci et al., 2012) and LRRK2 mutations (Santpere and Ferrer, 2009; Berwick and Harvey, 2013). Studies using 6-OHDA and MPTP PD models confirmed the dying-back phenomenon observed in PD. A retrograde degeneration of the nigrostriatal system was observed prior to the loss of nigral cell bodies in 6-OHDA rat model (Sauer and Oertel, 1994; Przedborski et al., 1995; Serra et al., 2002; Li et al., 2009a) and MPTP non-human primate (Herkenham et al., 1991; Meissner et al., 2003).

MPTP and $MPP^+$ treatments can induce a dysfunction and fragmentation of axonal microtubules (Cappelletti et al., 2005; Ren et al., 2005; Morfini et al., 2007). Midbrain mice culture treated with 6-OHDA showed the same pattern of fragmentation, in addition to a retrograde degeneration and the impairment of axonal transport (Lu et al., 2014). Neurotoxic agents directly bind to tubulin, which affects the tubulin polymerisation and microtubule transport leading to a axonal degeneration (Cappelletti et al., 1999, 2005).

About PD mutations such as α-synuclein mutation A30P and A53T in phosphorylation state have been shown to impair axonal transport and neurite outgrowth in rat primary midbrain and cortical neurons (Saha et al., 2004; Lee et al., 2006; Sousa et al., 2009; Prots et al., 2013; Tönges et al., 2014; Koch et al., 2015). The wild-type and mutated α-synuclein overexpression impairs microtubule polymerization and actin stability. Actin polymerization is regulated by Rho-associated protein kinase (ROCK) and its activation by α-synuclein mutations could block the neurite sprouting and induce a neurite retraction (see chapter: II. 6.2.3. RhoA pathway and ROCK inhibition) (Tönges et al., 2014).

Bacterial artificial chromosomes (BAC) transgenic LRRK2 mouse model of human mutated form (R1441G) shows a motor impairment but without loss of dopaminergic neurons (Li et al., 2009b). However, TH immunostaining of projection highlighted the dystrophic neurites and fragmented axons containing spheroid-like structures (Li et al., 2009b). LRRK2 mutation G2019S overexpressed in animals (rats, mice and drosophila) also induces neurite retraction, microtubule fragmentation, neurite inclusions and mislocalization of axonal protein tau (Lin et al., 2010a; Winner et al., 2011; Kawakami et al., 2012; Tsika et al., 2015). In agreement, studies in the SH-SY5Y neuroblastoma cells (Plowey et al., 2008; Chan et al., 2011) and primary hippocampal and midbrain cultures from mutant mice overexpressing the mutated G2019S form of LRRK2 (MacLeod et al., 2006; Dächsel et al., 2010; Ramonet et al., 2011; Sepulveda et al., 2013) showed a reduction of the neurite length and branching complexity. In contrast, these pathological changes were not observed after overexpression of wild-type LRRK2, whereas the LRRK2 downregulation increases the neurite length (MacLeod et al., 2006; Dächsel et al., 2010; Heo et al., 2010; Paus et al., 2013; Sepulveda et al., 2013). Mechanism leading to retraction induced LRRK2 mutations is not well understood but it seems closely related to phosphorylation status of the LRRK2 (Parisiadou and Cai, 2010; Greggio, 2012). The phosphorylated LRRK2 may in turn phosphorylate other proteins directly involved in neurite growth such as tau and β-tubulin as well as proteins involved in the signalling pathway of the polymerisation of microtubule and actin such as Rac1 (Parisiadou et al., 2009; Chan et al., 2011; Kawakami et al., 2012). Recent data showing the impact of LRRK2 on actin cytoskeleton guidance further support the interest of this protein in the dynamics and stability of the growth cone: crucial element for axon sprouting (Parisiadou et al., 2009; Häbig et al., 2013). The growth cone is a highly dynamic neuritic structure and essentially supported by a bundle of actin filament. It promotes for the generation of new

axonal and dendritic projection for the creation of the new synapses (Goodman, 1996; Lowery and Van Vactor, 2009; Kerstein et al., 2015).

## II. 6.2. Induction of dopaminergic differentiation and neurogenesis by transcription factors and its potential for the treatment of PD

Neurorestorative strategies for PD represents a large field of basic and pharmaceutical research. A huge number of promising targets have been proposed aiming the neurorestorative mechanisms including the activation of neurodifferentiation pathways towards the dopaminergic phenotype (Kalia et al., 2015). Among those attracting significant interests are the nuclear factors and the ras homolog gene family, member A (RhoA) downstream cell-signalling pathway because of their action on neuronal phenotype and the morphological neurodifferentiation respectively. Several transcription factors have been proposed as potential targets not only because of their potential restoration of dopaminergic phenotypes but also because of their subsequent neuroprotective component due to stabilization of neurotransmitter-specific synaptic elements. Nuclear factors such as retinoic acid receptor (RAR), liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR) and NURR1 are the more important targets, which, in most cases, act as heterodimer complexes with retinoic X receptor (RXR) binding to DNA sequences and inducing gene transcription (Skerrett et al., 2014).

### II. 6.2.1. Retinoic acid and RAR activation

Retinoic acid (RA), a metabolite of vitamin A, is major activator of RAR, potentially involved in the dopaminergic differentiation (Kitamura et al., 2002; Friling et al., 2009; Skerrett et al., 2014). RAR activation by RA plays an important role in the development of central nervous system and is mostly involved for midbrain dopaminergic neurons (Maden, 2007; Jacobs et al., 2011; Rhinn and Dollé, 2012; Allodi and Hedlund, 2014). Indeed, knockout mice of RAR have locomotor impairments and a reduction of D1R and D2R (Krezel et al., 1998). Moreover, a high expression level of RA synthesis enzyme is observed in the dopaminergic neurons (McCaffery and Dräger, 1994).

RA has been proposed as therapeutic option in PD due to its specific role in midbrain development, and its neuroprotective effect in several models of PD. RA reduces hydrogen peroxide-induced apoptosis (Moreno-Manzano et al., 1999; Kitamura et al., 2002). It has

been demonstrated that RA confers a higher resistance to $MPP^+$ and 6-OHDA treatment in SH-SY5Y cells and in rat primary ventro-mesencephalic cultures by upregulation of cell survival via activation of the Akt pathway (Cheung et al., 2009). RA also potentiates the synthesis of the nerve growth factor (NGF) and the expression receptor of neurotrophin trkA involved in the survival and development of neuronal cells, which could explain its protective effect (von Holst et al., 1995; Plum and Clagett-Dame, 1996; Arrieta et al., 2005). In PD models, RA has been shown to reverse the impairment of locomotor function and dopaminergic neuron degeneration induced by the 6-OHDA, MPTP and rotenone (Ulusoy et al., 2011; Yin et al., 2012; Esteves et al., 2015). Interestingly, pituitary homeobox 3 (PITX3) or NURR1 activation is proposed to participate to this neuroprotective effect of RA (Sacchetti et al., 2002; Jacobs et al., 2011; Hong et al., 2014). These transcriptional factors may act in cooperation and are crucial for the maturation and survival of the dopaminergic neurons (Jacobs et al., 2009, 2011; Hong et al., 2014). The deficient mice for PITX3 fail to develop dopaminergic neurons specifically in the substantia nigra and display a low level of dopamine and an impairment of locomotor function (Nunes et al., 2003; van den Munckhof et al., 2003; Smidt et al., 2004; Luk et al., 2013; Kim et al., 2014; Le et al., 2015). More interestingly, PITX3 directly activates the transcription of VMAT2 and DAT genes (Hwang et al., 2009).

The interest of NURR1 in PD has been strengthen by the proof-of-principle using genetic approaches (Zetterström et al., 1996; Wallen-Mackenzie et al., 2003; Hermanson et al., 2006; Park et al., 2006; Bae et al., 2009; Bensinger and Tontonoz, 2009; Decressac et al., 2013). NURR1 also up-regulates the expression of the TH, VMAT2 and DAT expression (Castillo et al., 1998; Hermanson et al., 2003; Kim et al., 2003; Smits et al., 2003). The NURR1-deficient mice display a reduction of striatal dopamine and of dopaminergic neuron markers in ventral midbrain and striatum, and a impairment of the locomotor function (Le et al., 1999; Jiang et al., 2005; Kadkhodaei et al., 2009). Histological analyses revealed a loss of dopaminergic neurons and an increased sensitivity to oxidative stress and to MPTP. A very recent study using bexarotene, an agonist of NURR1, reported a dopaminergic neuron protection and a reversion of the behavioural deficits in 6-OHDA PD model (McFarland et al., 2013). Authors suggested that the upregulation of DAT and VMAT2 expression modulated by NURR1 was probably implicated in the protective effect of bexarotene. These findings are also supported by reported data in rat embryonic stem cells and ventral midbrain cultures showing that XCT0139508, a NURR1 ligand, increases the dopamine cell survival

against 6-OHDA insult (Friling et al., 2009). Moreover, NURR1 transfection promotes neurogenesis in neuronal precursors from rat foetal cortices (Hermanson et al., 2006; Park et al., 2006; Bae et al., 2009; Jacobs et al., 2011; Hong et al., 2014).

RA induces neurogenesis and differentiation, an effect well characterized *in-vitro* models proposed for axonal regeneration (Puttagunta and Di Giovanni, 2011). RA-induced neurite growth and development of the growth cones have been observed in neuronal cell lines (SH-SY5Y) and in primary cultures from adult or embryonic rodent brains (Corcoran et al., 2000; Encinas et al., 2000; Presgraves et al., 2004; Dmetrichuk et al., 2006; Cheung et al., 2009; Farrar et al., 2009; Dwane et al., 2013; Korecka et al., 2013). RA-mediated differentiation in SH-SY5Y cells leads to a higher level of dopamine storage and expression level of dopaminergic markers such as TH or DAT (Hashemi et al., 2003; Presgraves et al., 2004; Constantinescu et al., 2007; Korecka et al., 2013). Overall results demonstrated the cell differentiation toward the dopaminergic phenotype. Currently, specific co-treatment with RA and other compounds has been used in embryonic stem cells to induce dopaminergic differentiation for grafting purposes in 6-OHDA PD model and also in PD patients (Fathi et al., 2010).

### II. 6.2.2. LXR nuclear factor

Nuclear receptor LXR regulates the level of cholesterol, fatty acid and glucose homeostasis (Wang et al., 2002; Skerrett et al., 2014). Besides this main role, LXR activation promotes neuron and midbrain neurogenesis (Wang et al., 2002; Andersson et al., 2005; Xu et al., 2014). The activation of LXR by oxysterol differentiates the embryonic ventral midbrain and mouse embryonic stem cells inducing higher levels of TH, VMAT2, PITX3, DAT and NURR1(Sacchetti et al., 2009; Theofilopoulos et al., 2013). LXR knockout mice present a higher sensibility to MPTP-induced degeneration of neuronal cells in substantia nigra (Dai et al., 2012). Moreover, T0901317 and GW3965, agonists of LXR, are able to recover motor function and to protect dopaminergic neurons against MPTP (Dai et al., 2012; Campolo et al., 2014). LXR upregulates another important transcription factor for regulation of the dopaminergic proteins called forkhead box protein A2 (FOXA2) (Arenas, 2008; Domanskyi et al., 2014). Suppression of FOXA2 gene triggers spontaneous parkinsonism with predominant loss of dopamine neurons of substantia nigra in mice (Kittappa et al., 2007). In a recent study, the injection of NURR1- and FOXA2-adenovirus markedly protects

the dopaminergic neurons and restores motor impairment in MPTP mice model (Oh et al., 2015).

### II. 6.2.3. RhoA pathway and ROCK inhibition

ROCK inhibition has been proposed as a potential target for PD (Tönges et al., 2011; Fujita and Yamashita, 2014; Labandeira-Garcia et al., 2014). ROCK is a kinase involved in the axonal growth and regeneration signalling. Two ROCK isoforms with a high homology have been identified: ROCK1 expressed in the periphery of the brain whereas ROCK2 expressed in central nervous system and in muscle (Tönges et al., 2011; Labandeira-Garcia et al., 2014). The direct endogenous activator of ROCK is the GTPase RhoA which is activated by signalling cascades coming from extracellular ligands such as Nogo, oligodendrocyte myelin glycoprotein (OMgp) or myelin associated glycoprotein (MAG) (Figure 7) (Cao et al., 2010; Saha et al., 2014; Seiler et al., 2015). These extracellular molecules are known to be synthetized by the oligodendrocytes, myelin, and scar tissue and represent a stopping signal for axonal growth though activation of complexes of Nogo receptor 1 (NgR1), LINGO-1 orp75NTR (Cao et al., 2010; Saha et al., 2014; Seiler et al., 2015). The RhoA activates the ROCK kinase activity by the disruption of the interaction between C- and N-terminal regions of ROCK and subsequently releases the auto-inhibition of ROCK (Tönges et al., 2011; Labandeira-Garcia et al., 2014). LIM domain kinase (LIMK) is the principal downstream effector of ROCK is (Yang et al., 1998; Bernard, 2007) and four other effectors were also described such myosin light chain (MLC), collapsing response mediator protein-2 (CRMP-2), ezrin, radixin and moesin (ERM) and adducin (Arimura et al., 2004; Kim and Chang, 2004). The activation of these downstream effectors leads to a stabilization and retraction of neurite, whereas its inhibition promotes neurite growth. Indeed, ROCK inhibition with fasudil and Y-27632 reduces the neurite growth in PC12, SH-SY5Y and primary neuronal neurons (Zhang et al., 2006; Ichikawa et al., 2008; Racchetti et al., 2010a; Tonges et al., 2012).

Phosphatase and tensin homolog (PTEN) has been identified as a downstream effector of ROCK (Li et al., 2005). PTEN is a phosphatase which negatively regulates PI3K/Akt pathway by the dephosphorylation of phosphatidylinositol(3,4,5)trisphosphate ($PIP_3$) in phosphatidylinositol 4,5-bisphosphate ($PIP_2$) (Gupta and Dey, 2012). PI3K/Akt has been identified as canonical pathway for the neuronal survival, the upregulation of protein synthesis and for the cell growth. Indeed, the activation of PI3K/Akt pathway through PTEN inactivation induced by ROCK inhibition protects against apoptotic cell death (Street and

Bryan, 2011). For exemplee, ROCK inhibitor Y-27632 is widely used for survival promotion in differentiation step of embryonic stem cell (Watanabe et al., 2007; Kamishibahara et al., 2014).

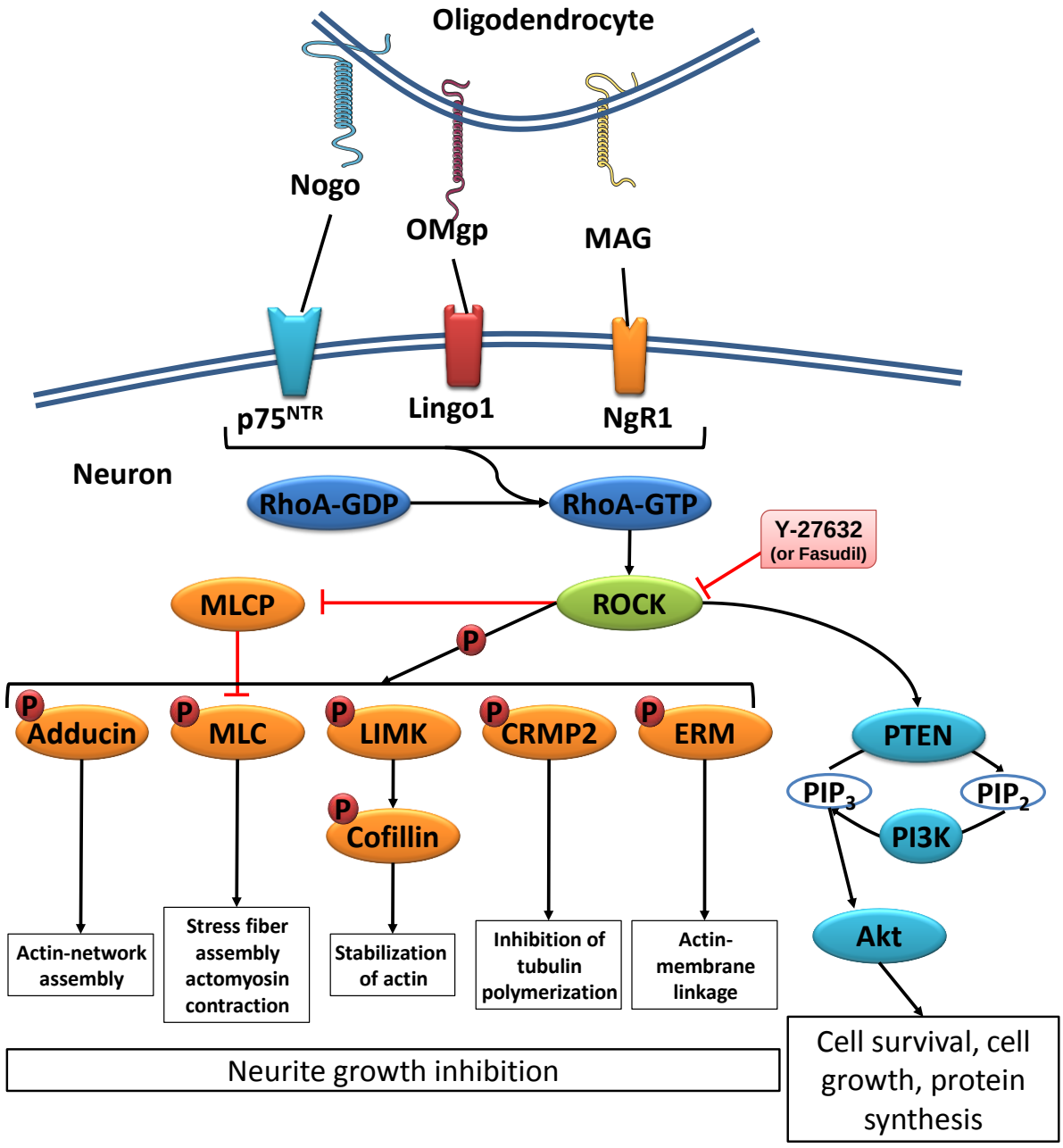


Figure 7. **ROCK signalling pathway involved in the regulation of neurite growth and cell survival.**

CRMP2, collapsin response mediator protein; ERM, ezrin, radixin and moesin; LIMK, LIM domain kinase; PTEN, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; p75NTR, p75 neurotrophin receptor; MAG, myelin-associated glycoprotein ; Nogo, reticulon-4; MLC, myosin light chain phosphatase; MLCP, myosin light chain protein; NgR, nogo Receptor; Omgp, oligodendrocyte-myelin glycoprotein; PI3K, phosphoinositide 3-kinase; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; $PIP_3$, phosphatidylinositol (3,4,5)-trisphosphate; ROCK, Rho-associated protein kinase;

ROCK inhibitors in PD may represent interesting disease-modifying principles for the treatment in PD due to the induction of neurite outgrowth and cell survival (Tönges et al., 2011; Labandeira-Garcia et al., 2014). Supporting this hypothesis, treatment of cells (SH-SY5Y, MES 23.5, rat primary midbrain and mesencephalic cultures) with Y-27632, fasudil or MBPTA reduce both death of TH positive dopaminergic neurons and axonal retractions induced by $MPP^+$ (Tonges et al., 2012; Borrajo et al., 2014). Animal studies also reported neuroprotective effects of fasudil or Y-27632 against dopaminergic neurons loss as well as restoration of the motor function in MPTP mice model (Tonges et al., 2012; Rodriguez-Perez et al., 2013; Zhao et al., 2015). The reported effect was associated to a reduction of proinflammatory activity (IL-1β, TNF-α, NF-κB-p65 and TLR-2) and oxidative stress (iNOS and gp91Phox). Moreover, downregulation of ROCK2 by viral small hairpin RNA (shRNA) transfection in primary midbrain neurons showed a strongest resistance to $MPP^+$ toxicity (Saal et al., 2015). The injection of this virus in mice treated with 6-OHDA leads to a decrease of loss of dopaminergic cells in substantia nigra, restoration of striatal TH fibers and motor improvement after 4 weeks.

Neurorestoration and differentiation could be mechanisms with therapeutic disease-modifying potential for the treatment of PD. The induction of neurorestoration by promoting both a dopaminergic phenotype and a growth of neurite and axons has been largely demonstrated for above mentioned pathways (Airavaara et al., 2012; Marxreiter et al., 2013; Hegarty et al., 2014). The phenotype induction could help to compensate the loss of dopaminergic neuron by a higher dopaminergic function of the remaining neurons and by the reduction of oxidative stress, while the growth of synaptic elements could regenerate dopaminergic terminals (Lingor et al., 2012; Burke and O'Malley, 2013; Lamm et al., 2014).

# III. Cholesterol, brain and statins

The inhibition of cholesterol biosynthesis has been proposed to have neuroprotective and neurorestorative effects. The following chapters outline how the statins, cholesterol-lowering drugs, acting at 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) may have a beneficial effect on neurological disorders including PD, and represent a potential disease-modifying principles for restoration of the dopaminergic terminals.

## III. 1. Cholesterol

Cholesterol is a hydrophobic molecule of the sterol family with intrinsic structural rigidity that plays an important role in the structure and function of cells (Yeagle, 1991, 1991; Krause and Regen, 2014). Cholesterol is essential for the growth and cell viability and represents 30% of total lipids of the human body (Krause and Regen, 2014). The cholesterol structure contains four carbon rings with an amphipathic component due to hydrophilic hydroxyl group on the carbon 3 and to the lipophilic hydrocarbon tail (Figure 8).

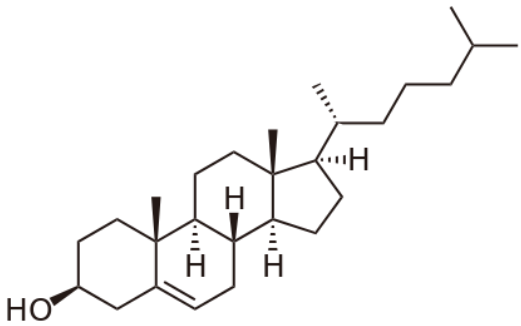


Figure 8. **The structure of cholesterol**

The cholesterol of mammalian cells is provided by dietary uptake (30%) while de novo cholesterol (70%) is mainly synthetized in the liver (Wilson and Lindsey, 1965; Wilson, 1972; Wilson and Rudel, 1994). Endogenous cholesterol is synthesized from glucose and fructose in the liver and extrahepatic tissues via the glycolysis and the mevalonate pathways (Popjak, 1958; Chang et al., 1997). Every cell has the capacity to synthesize the needed cholesterol and the body has various homeostatic mechanisms to regulate its levels (Phillips and Johnson, 1998).

The dietary cholesterol is transported to the liver by the lipoprotein chylomicrons while the synthesized cholesterol also uses the lipoprotein named VLDL (Figure 9) (very low-density lipoproteins). The lipoprotein particles are complexes of lipids and proteins that are more soluble in blood and lymph than free cholesterol due to amphipathic component of the cholesterol (Figure 10) (Myant, 1982; Lestavel and Fruchart, 1994; Goldstein and Brown, 2009). These lipoproteins represent the source of cholesterol for the muscles and fat cells. LDL (low-density lipoproteins) supply with cholesterol all other tissues (Myant, 1982; Lestavel and Fruchart, 1994; Goldstein and Brown, 2009). The transport of tissue cholesterol back to the liver happens by participation of HDL (high-density lipoproteins). The lipoproteins are trapped by the LDL receptor (LDLR), which leads to its endocytosis and digestion in several components including amino acids, cholesterol esters and free cholesterol by cell lysosomes (Myant, 1982; Lestavel and Fruchart, 1994; Goldstein and Brown, 2009).

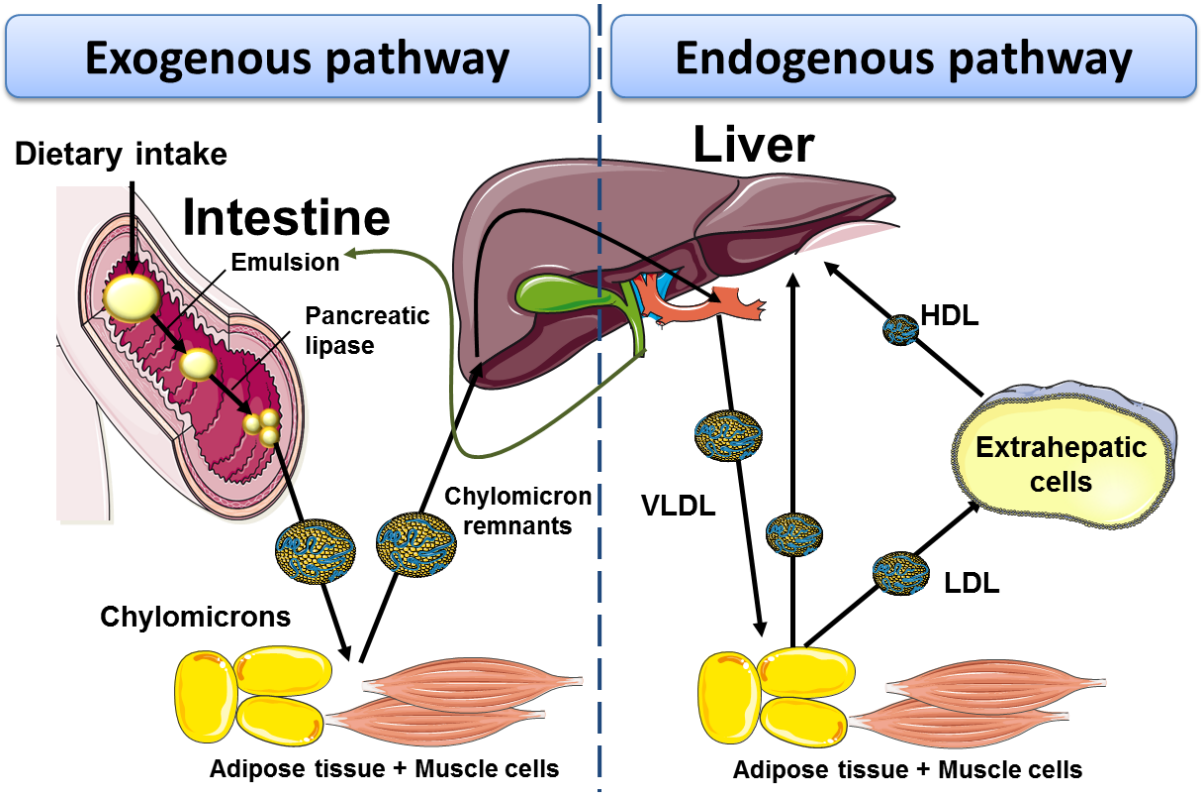


Figure 9. **Simplified cartoon of cholesterol lipoprotein particle transports between the intestine, liver and extrahepatic tissues**

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density liprotein.

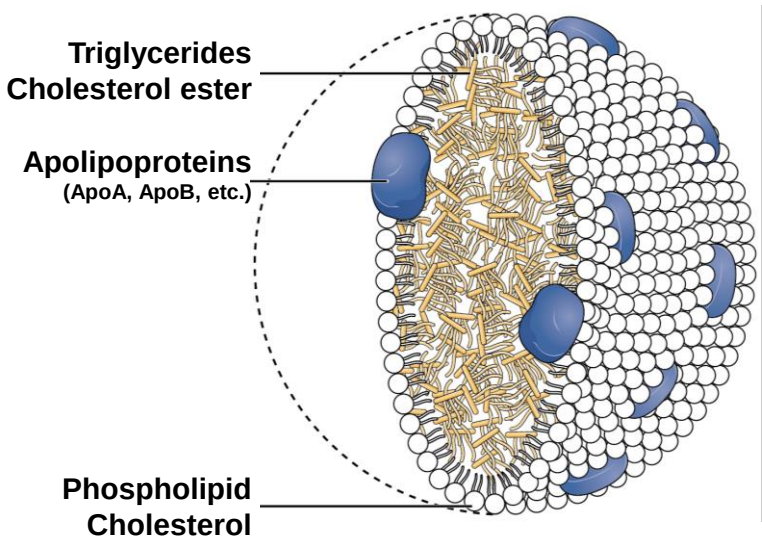


Figure 10. **Structure of the lipoproteins and the chylomicrons**



Cholesterol is abundant in both endosomes and Golgi apparatus, especially in the trans-Golgi zone while the endoplasmic reticulum contains only about 1% of cellular cholesterol (Ikonen, 2008). Cholesterol affects several cellular processes via its interaction with other membrane lipids and specific proteins and its structure increases the cohesion and the consolidation of the neighbouring lipids in the lipidic bilayer (Needham and Nunn, 1990) and subsequently a decrease in the fluidity and permeability (Cooper, 1978). Cholesterol orchestrates the formation of specialized areas (lipid rafts) in the plasma membrane by its lateral organization in the lipidic bilayer (Chong et al., 2009). Lipid raft containing the cholesterol with sphingolipids which has an affinity for proteins would be implicated in the signal transduction, membrane proteins and trafficking and cell adhesion (Simons and Ikonen, 1997; Simons and Ehehalt, 2002; Korade and Kenworthy, 2008; Pike, 2009). The biochemical organization of cholesterol allows to increase the mechanical strength of the lipid bilayer, which reduces the passive permeability of water, small molecules and gases (Róg et al., 2009). Cholesterol may also serve as precursor for steroid hormones and regulates the function of signalling molecules like hedgehog (Porter et al., 1996; Payne and Hales, 2004).

# III. 2. Brain cholesterol

The brain comprises 23% of the total body cholesterol at a concentration of 15-20 g/kg of brain tissue, essentially in a unesterified free form (99%) (Dietschy and Turley, 2001; Zhang and Liu, 2015). It is important to note that central nervous system represents only 2.1% of the body total weight, thus the brain is highly enriched in cholesterol compared to the rest of the body. All brain cholesterol is basically synthetized locally. In adult brain neurons are mostly dependent on astrocytes for its source of cholesterol via the lipoproteins containing the apolipoprotein E (ApoE) (Edmond et al., 1991; Dietschy, 2009; Pfrieger and Ungerer, 2011). However during embryogenesis, all cells produce a large amount of cholesterol needed for neurogenesis processes such as cell division and neurite formation (Dietschy and Turley, 2001; Dietschy, 2009). Brain cholesterol has a slow turnover rate unlike other tissues. However, brain astrocytes have a higher turnover rate of cholesterol synthesis allowing the transfer of a cholesterol amount for the neurons. The synthesis rate of brain cholesterol is only 0.9% of whole body, which translates into a half-life of 4-6 months in the rat and up to 5 years in a human brain (Björkhem et al., 1998; Dietschy and Turley, 2004; Vaya and Schipper, 2007). It also is important to highlight cholesterol from oligodendrocytes which represents 70% of total cholesterol in the brain (Björkhem and Meaney, 2004; Saher et al., 2005). These cells ensure neuronal transmission by formation of cholesterol-enriched myelin sheaths to wrap neuronal axon. Myelin sheaths reduce the permeability to ions, and therefore allow the propagation of the electrical impulse along the axon. Numerous other brain processes such as conformation of neurotransmitter receptors and transporters, synaptic vesicle microtubular transport and exocytosis fusion, cell adherent junctions, appropriate membrane curvature and assembly of vesicle-specific proteins and lipids are also dependent on the cholesterol (Yeagle, 1991; Koudinov and Koudinova, 2003; Pfrieger, 2003; Segatto et al., 2014). Today, it remains unclear what is role and how cholesterol level could affect the function, stability and biogenesis of the synaptic vesicles. However, cholesterol represents 40% of total lipids in synaptic vesicles (Takamori et al., 2006) and its interactions with the synaptic proteins (e.g. synaptophysin and synaptotagmin) have been reported (Thiele et al., 2000; He et al., 2006; Lv et al., 2008).

Brain cholesterol levels are tightly regulated because the cholesterol does not pass through the blood-brain barrier (Orth and Bellosta, 2012). This mechanism protects the brain of a too high level of plasma cholesterol and the subsequent deleterious effect of toxic free

cholesterol accumulation because of its amphipathic component (Björkhem and Meaney, 2004; Dietschy, 2009; Fantini and Yahi, 2015). Unlike other tissue, the cholesterol cannot be extruded of the brain and thus it is converted by the cholesterol 24-hydroxylase (Cyp46A1) in a form with polar component allowing its passage through the blood-brain barrier such as 24(S)-hydroxycholesterol and transport to the liver for degradation (Björkhem et al., 1998; Björkhem, 2006; Leoni and Caccia, 2013). Cholesterol 24-hydroxylase enzyme is highly expressed into brain and especially in the neuron in comparison to the rest of the body (Russell et al., 2009). 24(S)-hydroxycholesterol is able to regulate the cholesterol homeostasis of neurons and plays a critical role in cholesterol synthesis by the activation of LXR (see chapter: III. 3.2. SREBP regulation and cholesterol) (Björkhem, 2006; Cartagena et al., 2010; Gabbi et al., 2014).

# III. 3. Cholesterol synthesis and mevalonate pathway

The cholesterol synthesis involves the mevalonate pathway with a quite complex series of more than 25 separate enzymatic reactions. These reactions produce a range of different metabolites that regulate the mevalonate pathway (Buhaescu and Izzedine, 2007; Goldstein and Brown, 1990). The most critical steps of this pathway are summarized below (Figure 11).

The first step of the mevalonate pathway is the synthesis of HMG-CoA from three molecules of acetyl-CoA via two enzymatic reactions of acetoacetyl-CoA thiolase and HMG-CoA synthase (Massy et al., 1996; Buhaescu and Izzedine, 2007; Goldstein and Brown, 1990). Acetyl-CoA molecules are directly provided by oxidative conversion of pyruvate from the glycolysis pathway (Berg et al., 2002). Then, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) tightly regulates the conversion of HMG-CoA into mevalonate (Jo and Debose-Boyd, 2010). HMGCR is located in endoplasmic reticulum membrane and is the rate-limiting step enzyme of cholesterol synthesis (Friesen and Rodwell, 2004). In the absence of sterol into cells, HMGCR gene transcription is highly upregulated by the transcription factor sterol regulatory element-binding protein (SREBP) (Espenshade, 2006) (see chapter: III. 3.2. SREBP regulation and cholesterol). Following mevalonate synthesis, five subsequent reactions convert mevalonate into farnesyl pyrophosphate (FPP). The FPP is a pivotal substrate for two distinct pathways named prenylations (see chapter: III. 3.1. Prenylation pathway) (Miziorko, 2011; Holstein and Hohl, 2004). Two molecules of FPP are condensed

in squalene molecule by squalene synthase (Nes, 2011). This product is the first committed step of the specific cholesterol biosynthesis. Then, squalene is cyclized in two-step into lanosterol, which is in turn converted to cholesterol in a series of 19 reactions (Nes, 2011).

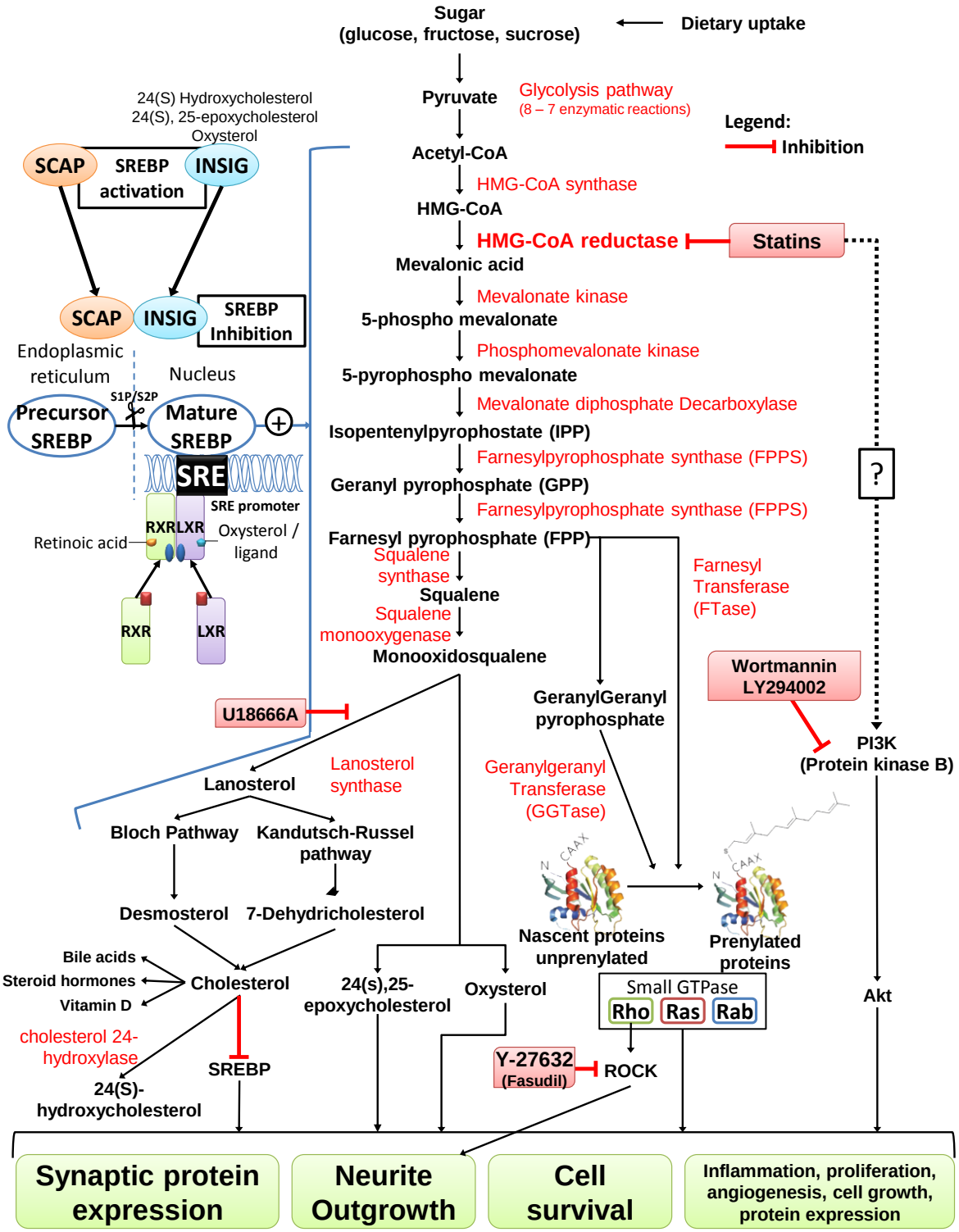


Figure 11. **Mevalonate pathway, regulation factors, isoprenoids, and cholesterol**

Insig, insulin-induced gene 1 protein; LXR, liver X receptor; ROCK, rho-associated protein kinase; RXR, retinoid X receptor; PI3K, phosphoinositide 3-kinase; S1P, site-1 protease; S2P, site-2 metalloprotease; SCAP, SREBP cleavage-activating protein; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein;

## III. 3.1. Prenylation pathways

The mevalonate pathway provides the essential isoprenoids lipids for the cells which are produced by the prenylation downstream pathways. Prenylation process is an important mechanism of post-translational modification of proteins and is required for proper subcellular localization and biological function of the proteins (Miziorko, 2011; McTaggart, 2006; Zhang and Casey, 1996). Farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) are the two enzymes that carry out the process of prenylation (Miziorko, 2011; McTaggart, 2006; Zhang and Casey, 1996). This process involves the covalent attachment of hydrophobic molecules to the cysteine motif CAAX (C, Cys; A, aliphatic amino acid; X, any amino acid) at the C-terminus of the proteins (Casey, 1992; Holstein and Hohl, 2004). A short-chain C-15 farnesyl pyrophosphate (FPP) or C-20 geranylgeranyl pyrophosphate (GGPP) are attached to the proteins by the enzymes GGTase and FTase respectively (Casey, 1992; Holstein and Hohl, 2004). Both enzymes preferentially attach isoprenoids to the small GTPases such as Ras, RhoA, and Rac families, which enables the protein anchorage into lipid membrane. This anchorage in membrane of the small GTPases acts as molecular switches for the regulation of their respective signalling pathway such as cell proliferation, inflammation and oxidative stress (Park et al., 2014; Li et al., 2012; Hooff et al., 2010; Palsuledesai and Distefano, 2015; Schafer and Rine, 1992).

## III. 3.2. SREBP regulation and cholesterol

The major feedback control of cholesterol synthesis is performed by the family of SREBP transcriptional factors (Espenshade, 2006; Shao and Espenshade, 2012). SREBPs enhance the transcription of more than 30 genes including proteins for uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids (Espenshade, 2006; Ye and DeBose-Boyd, 2011; Shao and Espenshade, 2012). Two genes encode three SREBP isoforms: SREBP-1a, SREBP-1c and SREBP-2. SREBP-1a is a potent activator of all SREBP-responsive genes, including the gene transcriptions for the synthesis enzymes of fatty acids and cholesterol (Horton et al., 2002; Ferré and Foufelle, 2007). SREBP-1c preferentially enhances the transcription of genes encoding for enzyme of the fatty acid synthesis, whereas SREBP-1a essentially increases the transcription of genes involved in the cholesterol synthesis (Horton et al., 2002; Ferré and Foufelle, 2007). SREBP-1c is predominant within the liver and other tissues but the transcriptional activity of this isoform is weaker than SREBP-1a. SREBP2 is ubiquitous with the same amount as the total amount

of SREBP-1a and -1c. SREBPs are anchored in the endoplasmic reticulum (ER) (Figure 12) (Bengoechea-Alonso and Ericsson, 2007; Sato, 2009; Eberlé et al., 2004). The high cytosolic cholesterol level sequesters SREBPs in the ER, while low cholesterol level leads to the transcriptional activity of SREBPs. SREBPs contain two distinct regulatory domains, the C-terminus forming a complex with SREBP-cleavage-activating protein (SCAP) and the N-terminus, a basic helix-loop-helix leucine zipper transcription domain necessary for transcription activation (Gasic, 1994; Yokoyama et al., 1993). SCAP is able to detect the cholesterol level via its "sterol-sensing" domain. Low cholesterol level leads to a conformational change of SCAP that promotes the release of the protein Insig (Sato, 2010; Shimano, 2001; Brown and Goldstein, 1997) and allows the transport of this complex in COPII-coated vesicles to the Golgi (Edwards et al., 2000; Wang et al., 1994; Xiao and Song, 2013). In the Golgi, SREBP is processed by two sequential proteolytic cleavages, which releases the N-terminus transcription factor domain (Bengoechea-Alonso and Ericsson, 2007; Sato, 2009; Eberlé et al., 2004). First, the site-1 protease (S1P) cleaves the consensus sequence RxxL (Arg-X-X-Leu) in the luminal loop, then site-2 metalloprotease (S2P) cleaves the intramembrane sequence of SREBPs in the lipid bilayer releasing the N-terminus (Espenshade, 2006). A dimer of the cleaved N-terminus domain of SREBP is formed into the cytosol and then it is transported into the nucleus by importin-β (Lee et al., 2003). The transcription of lipid metabolic genes is activated by binding to the sterol regulatory element (SRE) promoter (Espenshade, 2006).

The SREBP-1c gene is regulated by the nuclear transcription factor LXR. (see chapter: II. 6.2.2. LXR nuclear factor) (Repa et al., 2000; Yoshikawa et al., 2001). Oxysterol, hydrocholesterol and demosterol families are identified as potential activator ligands such as 25-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S),25-Epoxycholesterol, etc. (Schultz et al., 2000; Xiao and Song, 2013). The activation of LXR leads the regulation of LXRE promoter upstream to SRE promoter and also activates the genes of lipid metabolism specific of SREBP-1c (Yoshikawa et al., 2001).

Recently, studies showed a link between the PD and SREBP. A genome-wide association study on PD cohort has identified a SNP for SREBP1 gene and more especially for gene coding for SBREP-1c splicing form (Do et al., 2011). The genome-wide RNAi screen study demonstrated that SREBP1 gene plays a conserved role in mitophagy. SREBP-1 could stabilize PINK1 (PARK6) during the initiation of mitophagy (Ivatt et al., 2014).

Genistein, a blocker of SREBP activation by S1P inhibition, blocks the mitochondrial translocation of the E3 ubiquitin-protein ligase (PARK2, parkin). This effect is also observed with SREBP1 downregulation with siRNA (Ivatt and Whitworth, 2014).

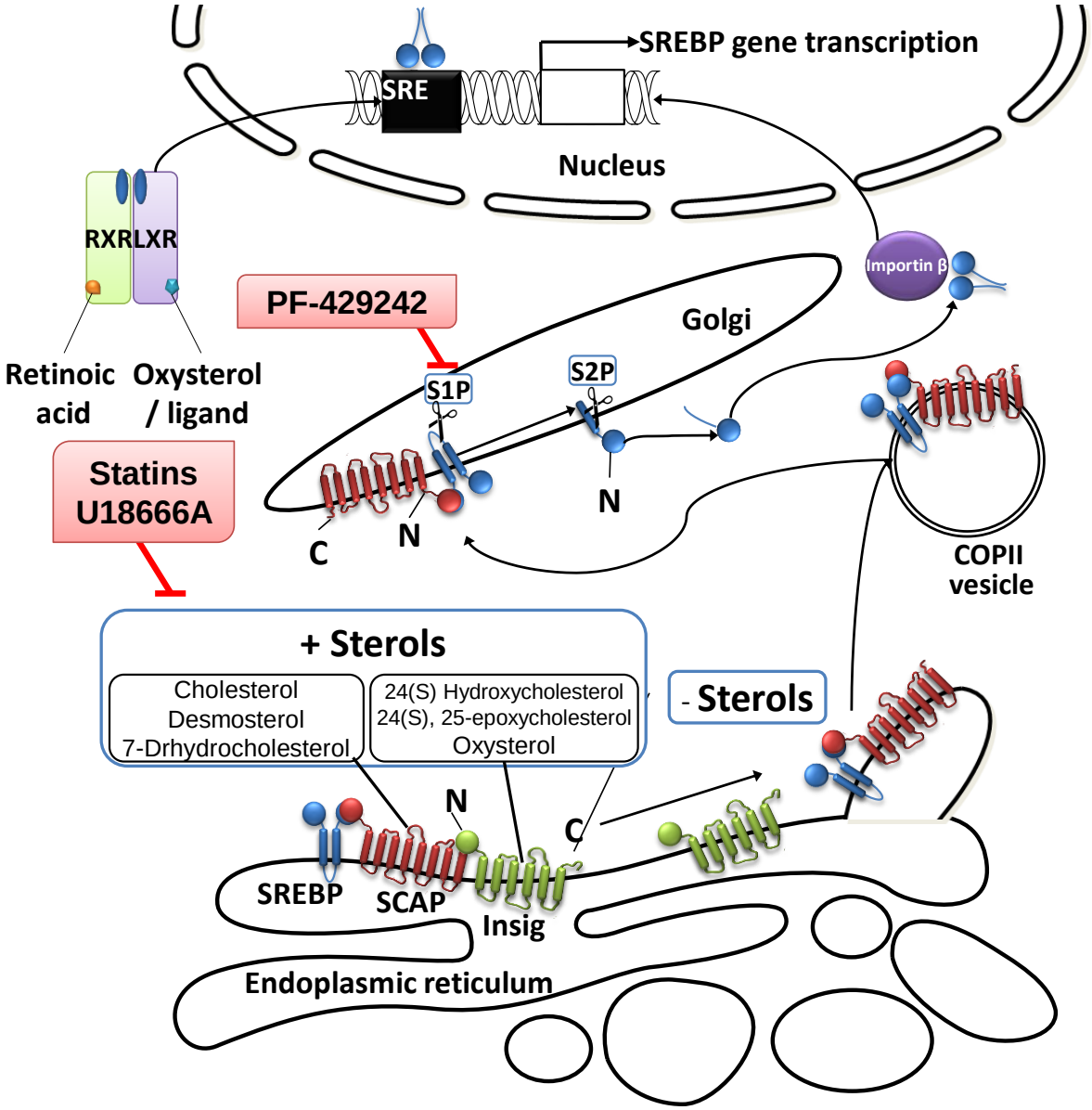


Figure 12. **The pathway of SREBP activation in the cholesterol metabolism**

Insig, insulin-induced gene 1 protein; LXR, liver X receptor; RXR, retinoid X receptor; S1P, site-1 protease; S2P, site-2 metalloprotease; SCAP, SREBP cleavage-activating protein; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein;

## III. 3.3. Statins in diseases

Statins are the main pharmaceutical drugs used for cholesterol. Mevastatin was the first statin identified by Endo and colleagues in 1976 and extracted from Penicillium Citrinium fungi (Figure 13) (Endo et al., 1976; Alberts, 1988). Later in 1980s and 1990s, several other statins were either extracted from other fungi or they were chemically modified such as lovastatin, simvastatin, atorvastatin, fluvastatin, pravastatin and cerivastatin. These new drug entities display a higher selectivity, affinity, lipophilicity and efficacy in patients and they are less hepatotoxic than mevastatin (Alberts et al., 1980; Alberts, 1990; Illingworth and Tobert, 1994; Jones, 2003). Statins are widely used to diminish the risk of occurrence (primary prevention) and recurrence (secondary prevention) of cardiovascular diseases resulting from the accumulation of cholesterol such myocardial infarction, angina pectoris, peripheral artery disease, stroke. They prevent artery narrowing and occlusion by atherosclerotic plaque rupture (Fuster et al., 1990; Palinski and Napoli, 2002; Taylor et al., 2013). Thus, cardiovascular morbidity and mortality are strongly reduced with statins (Taylor et al., 2013; Mihos et al., 2014; Prasad, 2014). The target of statins is the competitive inhibition of the conversion of HMG-CoA to mevalonate by HMGCR (Goldstein and Brown, 1990). Statins block a portion of the HMG-CoA catalytic binding site of the HMGCR (Istvan and Deisenhofer, 2001; Tabernero et al., 2003). Statins act principally by the inhibition of the HMGCR cholesterol production but also by an increase in the expression of cell surface LDLR via the SREBP activation (Tobert, 2003; Sirtori, 2014). The LDLR overexpression increases the clearance of LDL cholesterol from the blood flow ranging of 30% to 50% (Ma et al., 1986). In addition, based on recent preclinical and clinical researches, many beneficial pleiotropic effects in cardiovascular diseases are attributed to the statins which are independent of the cholesterol reduction (Liao and Laufs, 2005; Satoh et al., 2015; Profumo et al., 2014; Takemoto and Liao, 2001). Indeed, by inhibition of mevalonate synthesis, statins also inhibit the prenylation pathways and subsequently modulates the activity of the small GTPases involved in anti-inflammation, anti-oxidant and anti-thrombotic effects (see chapter: III. 3.1. Prenylation pathway). To summarize, statins reduce the inflammation and oxidative stress and activate the angiogenesis, endothelial nitric oxide synthase (eNOS) expression and circulating endothelial progenitor cells (EPCs). Thus, endothelial blood cells increase their capacity of nitric oxide production, which results in a reduction of atherogenic process due to an inhibition of the platelet aggregation in the thrombus, smooth muscle contraction and

endothelial-leukocyte interactions (Liao and Laufs, 2005; Satoh et al., 2015; Profumo et al., 2014; Takemoto and Liao, 2001).

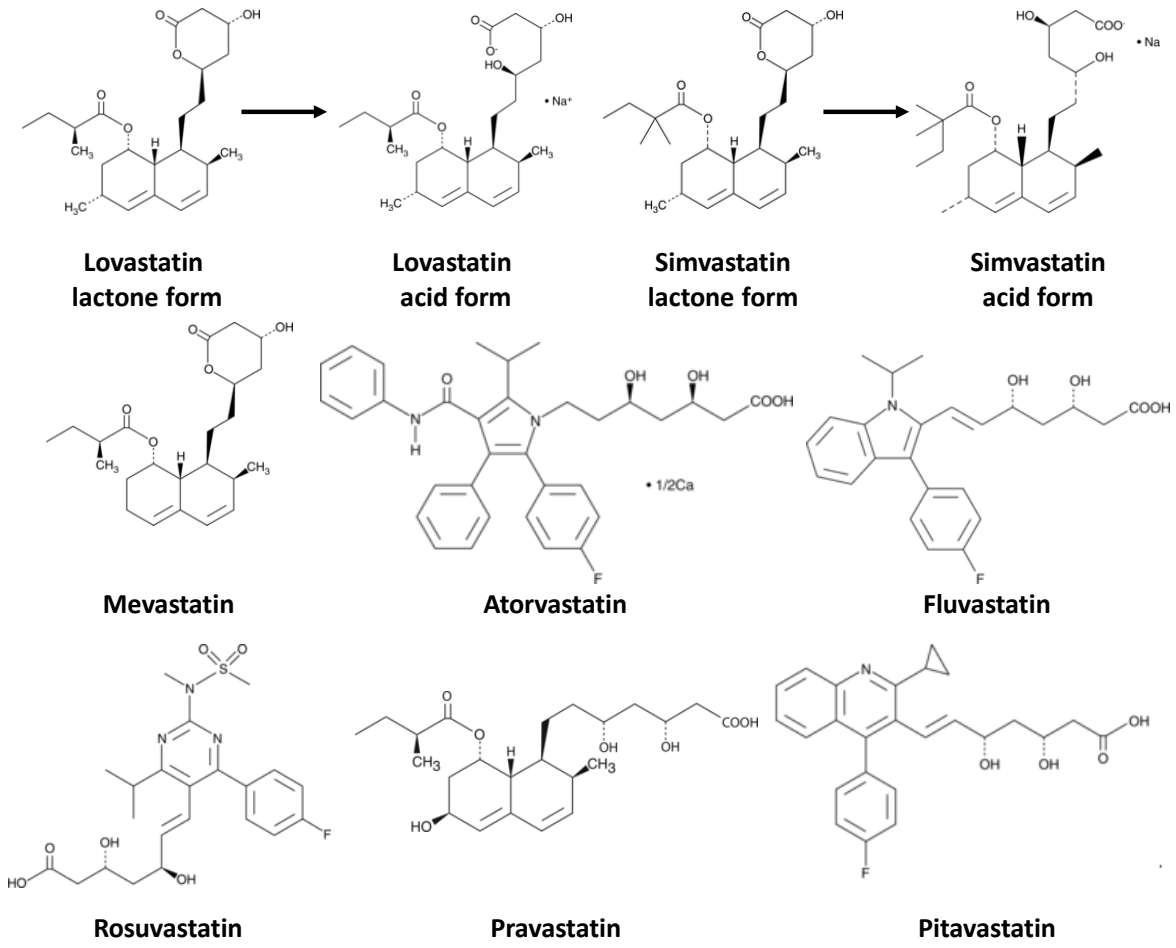


Figure 13. **Chemical structures of the statins**

During the last two decades, statins have been extensively studied in other diseases such as autoimmune diseases (e.g. rheumatoid arthritis) and in the neurological disorders such as stroke, multiple sclerosis, dementia, Alzheimer's disease and PD (Willey and Elkind, 2010; Silva et al., 2013; McFarland et al., 2014; Roy and Pahan, 2011; Wood et al., 2014; Reiss and Wirkowski, 2007; van der Most et al., 2009; Wang et al., 2011; Malfitano et al., 2014). The inhibition of prenylation pathways are proposed to mediate the pleiotropic neuroprotective effects in PD as proposed for the additional protective effect in cardiovascular diseases. In addition to the inhibition of prenylation, the activation of PI3K/Akt pathway by statins is commonly accepted by the research community and could contribute to the pleiotropic effects. However, the exact pharmacologic mechanisms leading

to neuroprotective remains poorly studied and could be related to the pleiotropic effects, but no consensus was found for neuroprotective effect of statins in PD.

## III. 3.4. Statins and phosphoinositide 3-kinase/AKT pathway

Many studies suggest that statins activate PI3K/Akt pathway while the mechanism is not identified. Indeed, statins promote rapidly and effectively the activation and phosphorylation of PI3K and Akt in *in-vitro* and *in-vivo* experimental models (Zhang et al., 2007; Wu et al., 2008; Jin et al., 2012; Yang et al., 2012). PI3K mediates the activation of serine/threonine protein kinase AKT and involves at least three subunits Akt1/PKBa, Akt2/PKBb and Akt3/PKBg (Manning and Cantley, 2007; Jha et al., 2015; Hemmings and Restuccia, 2012; Cantley, 2002). This PI3K/Akt signalling pathway is usually activated by neurotrophic factors such as platelet derived growth factor receptor (PDGF-R), insulin, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor I (IGF-I). PI3K is a family of intracellular lipid kinases classified in groups (class I, II, III) and class according to signalling receptor and the substrate specificity for phosphatidylinositols (Manning and Cantley, 2007; Jha et al., 2015; Hemmings and Restuccia, 2012; Cantley, 2002). To summarize, most of phosphatidylinositols (PI and PIP2) are converted by PI3K to PIP3 at the plasma membrane. The PIP3 recruits and activates the Akt in multistep process with the coupling of 3-phosphoinositide-dependent kinase 1 or 2 (PDK1 or PDK2) (Manning and Cantley, 2007). The activation of Akt activates or inhibits, by phosphorylation, potentially thousands of cellular substrates, more than 50 of them have been characterized so far (Manning and Cantley, 2007). Obviously, here, it is difficult to present all of them, but they are involved multiple cellular processes such as the regulation of cell survival, proliferation and insulin-dependent metabolic cell responses (Wang et al., 2012; Yang et al., 2004; Matsuda et al., 2013; Song et al., 2005; Shanware et al., 2013; Carracedo and Pandolfi, 2008). It has also been demonstrated that this pathway is implicated in the survival of neurons and in the reduction of neurological disorders (Jha et al., 2015; Kitagishi et al., 2012; Uranga et al., 2013; Brunet et al., 2001; Datta et al., 1999).

# III. 4. Statins in Parkinson's disease

## III. 4.1. Cholesterol and statins in epidemiological Parkinson's disease studies

Epidemiological studies highlighted the potential association between plasma cholesterol levels and the risk of PD. Higher occurrence of PD is associated with low LDL concentrations in a case-control study (LDL-C ≥ 138 mg/dL, OR 2.2 vs LDL-C to 115 to 137 mg/dL, OR 3.5 vs LDL-C of 92, OR 2.6) (Huang et al., 2007). Four prospective and retrospective population-based studies also found a statistically significant relationship between lower VLDL, LDL, HDL and triglyceride levels and an increased risk of PD (de Lau and Breteler, 2006; Huang et al., 2008; Wei et al., 2013; Guo et al., 2014). Reciprocally, large prospective study (121,046 women; 50,833 men) reported an association between a self-reported history of hypercholesterolemia and a lower PD risk (OR 0.86) (Simon et al., 2007). In addition, a large prospective population-based Rotterdam study based on primary care database with 6,465 subjects demonstrated a link between high levels of cholesterol and a decreased risk of PD (OR 0.77) (de Lau and Breteler, 2006; Becker and Meier, 2009). In contrast, high total cholesterol is associated with an increased risk of PD in a large Finnish population-based prospective (total cholesterol < 5, 5–5.9, 6–6.9, and ≥ 7 mmol/L, respective OR 1.00, 1.42, 1.56 and 1.86) (Hu et al., 2008). Overall, an association between cholesterol levels and PD risk might still be inconclusive due to contradictory finding.

Retrospective epidemiological study (312 PD patients and 342 controls) showed an inverse association between the use of statins and the risk of PD in a dose-response relationship (OR 0.37) (Wahner et al., 2008). The prospective epidemiological data analysis of register-based from 4.5 million of United-State veterans demonstrated a significant inverse association between the use of simvastatin and the risk of PD (OR 0.46) whereas atorvastatin is associated to a modest reduction of PD (OR 0.91) (Wolozin et al., 2007). More recently, a large study among 43,810 users of statins also showed a decreased of the risk of PD with lipophilic statins treatment such as simvastatin (lipophilic statins: OR 0.42 and simvastatin: OR 0.23), especially in female users (OR 0.11). But, atorvastatin had a lower significant effect (OR 0.42) (Lee et al., 2013). During 12 years of a follow-up study of 38,192 men and 90,874 women, the risk of PD was lower among current statin users compared to the control group (OR 0.74) but only in patients aged less than 60 years (< 60 years: OR 0.31 vs > 60

years: OR 0.83) (Gao et al., 2012). Finally, the meta-analysis of eight studies support the hypothesis that statin use reduces the risk of PD (OR 0.77) (Undela et al., 2013).

Different results have been found in epidemiological studies, certainly due to the study-related factors such as metholody, population sizes, PD diagnosis, type and dose of statins (Table 7) (Reiss and Wirkowski, 2007; Willey and Elkind, 2010; Dolga et al., 2011; Roy and Pahan, 2011; Wang et al., 2011; Malfitano et al., 2014).

**Table 7. Statins - general and clinical pharmacology - Parameters related to blood-brain barrier penetration and lipophilicity**

| | Pharmacodynamics - pharmacokinetics pharmacology parameters | | | | | | | | | Brain pharmacokinetics - permeability | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Statins** | **$IC_{50}$ HMG-CoA reductase (nM)** | **Oral absorption (%)** | **Bioavailability (%)** | **Liver extraction (%)** | **Protein binding (%)** | **Half-life (t1/2) (h)** | **Metabolism CYP450** | **Statins transporter involved** | **Standard daily dose (mg)** | **LogP** | **PAMPA assay (% crossing)** | **Cholesterol levels after 1µM statins treatment (% related untreated cells)** | | | **Rat blood-brain-barrier permeability (µL/min/cm²) [14C]-statins** | | | | **LogD coefficients** | |
| | | | | | | | | | | | | **Neuron cells (SK-N-MC)** | **Glial cells (U87MG)** | **Hepatic cells (Hep-G2)** | **Lactone form** | **Acid form** | **Lactone form** | **Acid form** | **Lactone form** | **Acid form** |
| **Pravastatin** | 4 | 35 | 18 | 45 | 50 | 1-3 | 3A4 | OATPB1 / MRP2 | 10-40 | 3.06 | 0 | 89 | 103 | 107 | 0.178 | | 0.0755 | | 2.42 | -0.47 |
| **Lovastatin** | 3 | 30 | 5 | 70 | >98 | 2-5 | 3A4 | OATP1B1 | 10-40 | 4.09 | 10.8 | 73 | 100 | 88 | 57.9 | 0.653 | 8.32 | 0.193 | 3.91 | 1.51 |
| **Simvastatin** | 1.2 | 72 | 5 | 80 | >95 | 2-5 | 3A4 | MRP2 | 10-40 | 4.63 | 33 | 47 | 72 | 91 | 27.7 | 0.442 | 4.76 | 0.42 | 4.4 | 1.88 |
| **Fluvastatin** | 6.5 | 98 | 30 | 70 | >98 | 1-3 | 2C9 | OATP1B1 | 80 | 4.18 | 28 | 55 | 92 | 91 | n/d | | | | 3.65 | 1.75 |
| **Atorvastatin** | 1.16 | 30 | 12 | 70 | >98 | 7-20 | 3A4 | OATP1B1 | 10-80 | 5.55 | 4.9 | 59 | 103 | 90 | | | | | 4.4 | 1.53 |
| **Rosuvastatin** | 0.16 | 50 | 20 | 63 | 90 | 20 | 2C9 | OATP1B1 | 5-40 | 2.29 | 0.1 | 63 | 91 | 87 | | | | | n/d | |
| **Pitavastatin** | 0.1 | 80 | 60 | n/d | 96 | 10-13 | 2C9 | OATPB1 / MRP2 | 1-4 | 4.58 | 12.7 | 60 | 92 | 87 | | | | | | |

LogP, partition-coefficient and LogD: distribution-coefficient (D): ratio of concentrations of statin in a mixture of two immiscible phases at equilibrium (octanol/eau). These coefficients are a measure of the difference in solubility and lipophilicity. LogD take into account the pKa of the compound

PAMPA: parallel artificial membrane permeability assay, determines the permeability of substances from a donor compartment through lipid-infused artificial membrane into an acceptor compartment

Adapted from (Hsiang et al., 1999; van der Most et al., 2009; Sierra et al., 2011; Wood et al., 2014). n/d, not determine.

# III. 4.2. Statins and their effects in Parkinson's disease models

## III. 4.2.1. Neuroprotective effects of statins

### III. 4.2.1.1. Inhibition of proinflammatory process

Statins could modulate the proinflammation process though the PI3K/Akt and prenylation pathways (Greenwood et al., 2006; Kitagishi et al., 2012). In PC12 cells, simvastatin treatment protects cells against 6-OHDA toxicity and downregulates the proinflammatory factors such as TNF-α, IL-6 and COX-2, probably though the activation of PI3K/Akt pathway (Xu et al., 2013). Moreover, this reduction of proinflammatory activation as well as the protective effect are confirmed in two other studies by measuring the mRNA reduction of the TNF-α, IL-1β, and IL-6 and MMP9 in PC12 treated with 6-OHDA (Yan et al., 2014a, 2014b). In lipopolysaccharide (LPS)–stimulated rat primary astrocytes, lovastatin is able to inhibit the activation of NF-κB, the expression of iNOS and proinflammatory cytokines (TNF-α, IL-1β, and IL-6) (Pahan et al., 1997). In addition, simvastatin inhibits the production of TNF-α and nitric oxide in cultured rat microglia stimulated with the LPS (Selley, 2005). The reduction of iNOS and the inhibition of NF-κB triggered by statins can be reverted with the addition of mevalonate or FPP, whereas the proinflammation inhibition effect with lovastatin remains unchanged with the cholesterol treatment (Pahan et al., 1997). Overall, these results suggest the involvement of prenylation pathway rather than cholesterol pathway in the reduction of proinflammatory response by the statins. Moreover to support previous data, the FTase inhibitors suppress also the activation of NF-κB and expression of iNOS induced by the LPS treatment in glial cells (Pahan et al., 1998, 2000). In addition, simvastatin can block the activation NF-κB and $p21^{Ras}$ (a apoptotic and proinflammatory factor) induced with $MPP^+$ in microglia (Ghosh et al., 2009). In MPTP treated mice, simvastatin is able to strongly inhibit the synthesis of proinflammatory components and the activation of $p21^{Ras}$ the substantia nigra (Ghosh et al., 2009). Moreover, TNF-α and IL-6 is reduced by simvastatin and atorvastatin treatments in the 6-OHDA PD model (Kumar et al., 2012). Together, these studies suggest that statins are capable to suppress the expression of proinflammatory cytokines, chemokines, adhesion molecules in glial cells and PD models via attenuation of prenylation and/or PI3K/Akt pathways. Thus, the anti-inflammatory effect of statins may be beneficial for neuroprotection in PD patients (van der Most et al., 2009)

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#### III. 4.2.1.2. Reduction of oxidative stress

Some evidence suggests that statins induce a reduction of oxidative stress. Simvastatin inhibits the formation of ROS such as 3-nitrotyrosine in MPTP-treated mice. Statins inhibit geranylgeranylation of Rac and thereby can attenuate the NADPH oxidase-mediated generation of ROS (Di-Poï et al., 2001; Miyano and Sumimoto, 2012). In 6-OHDA treated rat, atorvastatin and simvastatin are able to decrease the glutathione which is an antioxidant produced in ROS oxidative stress conditions (Kumar et al., 2012). Thus, the decreased of glutathione suggests a reduction of oxidative stress induced by the ROS. Supporting this data, lipid peroxidation referring to the oxidative degradation of lipids is reduced by atorvastatin. Therefore, statins may provide neuroprotection in PD by inhibiting NADPH oxidase, lipid peroxidation and ROS (Zacco et al., 2003; Reiss and Wirkowski, 2009; Ramirez et al., 2011).

#### III. 4.2.1.3. Protection against neurotoxic agents

In MPTP treated mice, simvastatin restores the level of biogenic amines (dopamine, DOPAC and HVA) in the striatum, independently of the brain cholesterol level. Moreover, high dose of simvastatin is able to produce more biogenic amines than the control (Selley, 2005). In MPTP-intoxicated mice, simvastatin and pravastatin improve motor functions (Ghosh et al., 2009). In contrast, other studies fail to demonstrate a neuroprotective effect of simvastatin against MPTP-induced death of dopaminergic neurons (Santiago et al., 2009). Moreover, fluvastatin or pitavastatin treatments display no significant changes in the neuroprotection in the MPTP model (Yokoyama et al., 2008). However, in 6-OHDA rat model, atorvastatin and simvastatin enhance mitochondrial complex I and III activity and restore locomotor activity, which increases the cell survival (Kumar et al., 2012). The protective effect of simvastatin in 6-OHDA model has been confirmed by the observation of a higher proportion of TH positive cells in the substantia nigra and by the restoration of expression level of NMDA receptor measured by the autoradiography binding of [$^3$H]MK-801 (Yan et al., 2011a). Lovastatin also protects against glutamate-induced toxicity in primary cortical neurons by activating the TNF receptor 2 (TNFR2) signalling pathway which is involved in the activation of PI3K/Akt pathway (Zacco et al., 2003; Dolga et al., 2008). In addition, lovastatin rescues cholinergic neuron and projection fibers after NMDA infusion injury in rodents. The PI3K/Akt pathway is proposed to mediates this protective effect (Dolga et al., 2009).

#### III. 4.2.1.4. Inhibition α-synuclein aggregation

Statins induce a redistribution of α-synuclein in caveolar fractions (a special type of lipid raft) in primary neuronal culture and decrease the α-synuclein accumulation and oxidation (Bar-On et al., 2008). In contrast, cholesterol supplementation promotes α-synuclein aggregation and reduces the neuronal outgrowth (Fortin et al., 2004). More importantly, lovastatin has also been investigated *in-vivo* mice overexpressing α-synuclein (Koob et al., 2010). A high lovastatin dose in these mice reduces significantly the oxidized cholesterol metabolites involved in the aggregations of α-synuclein. Thus, lovastatin reduces the neuronal degeneration and restores the locomotor dysfunction in mice overexpressing α-synuclein. Moreover, the reduction of oxidative stress by statin could also help to reduce α-synuclein aggregation (Esteves et al., 2009). Bosco and colleagues suggest that the oxidized cholesterol accelerates α-synuclein fibrillization and it is accumulated in the Lewy bodies, thus, it could contribute negatively to α-synucleinopathy (Bosco et al., 2006).

#### III. 4.2.1.5. Endothelial nitric oxide synthase (eNOS) activation

eNOS activation could be beneficial for PD by the capacity of eNOS to produce endothelium-derived nitric oxide (Tieu et al., 2003). It increase blood flow and endothelium relaxation in the brain (Dimmeler et al., 1999). The eNOS knockout mice are more sensitive to MPTP intoxication than the wild-type mice. However, currently, no data are available on the relationship between eNOS and PD. As mentioned before, statins reduce the expression of iNOS, while the opposite effect is found for eNOS associated to higher production of NO (Hernández-Perera et al., 2000). GGPP but not FPP is able to abolish the induction of eNOS by the statins suggesting a RhoA involvement in statin-induced eNOS (Hernández-Perera et al., 1998). However, Fulton and colleagues demonstrated that the PI3K phosphorylates eNOS and increases the production of nitric oxide (Fulton et al., 1999). Thus, statins may upregulate eNOS via the RhoA or PI3K/Akt pathways, which could have beneficial effect in PD patients.

#### III. 4.2.1.6. Modulation of synaptic proteins

The treatment of 6-OHDA deregulates the expression and function of D1R and D2R in the striatum (Qin et al., 1994). In this context, simvastatin increase the level of D1R and D2R in normal condition but also restores the level both receptors in 6-OHDA-induced PD rats (Wang et al., 2005). In the same condition, brain tissue from striatum treated with

simvastatin presents higher-level of dopamine content up to 110%. However, dopamine uptake remains unchanged in the synaptosomal preparation (Wang et al., 2006). Regarding other synaptic markers, the reduction TH and DAT expressions induced 6-OHDA treatment levels are partially reverted by simvastatin in PC12 cells (Xu et al., 2013). Besides the activation of lipidic and metabolic gene expressions, the chronic administration of lovastatin, pravastatin and simvastatin can upregulate the gene expressions of many other cellular processes (Johnson-Anuna et al., 2005; Dong et al., 2009). Interestingly, lovastatin treatment enhances the expression of SV2A more than 10 fold in non-neuronal HepG2 cells. This result correlates with the transcriptional upregulation of SV2A up to 40 fold induced by SREBP-1 overexpression in HepG2 and human fibroblast AG01518 cells transfected (Kallin et al., 2007). In addition, downregulation of SREBP-2 with shRNA lentivirus infection reduces the intensity of the postsynaptic density 95 (PSD95) and synaptobrevin expression levels in dendrites of hippocampal neurons (Suzuki et al., 2010).

### III. 4.2.2. Neurorestoration effects of statins

Statins are also investigated for their disease-modifying potential in several neurological disorders such as epilepsy, multiple sclerosis and Alzheimer's disease (Dimmeler et al., 2001; McGuinness et al., 2014; Malfitano et al., 2014). In stroke model, statins are associated to a potential dual effect of neuroprotection and neurorestoration during and after ischemic injury respectively. It has been suggested that statins may improve neurological function in a rat model of stroke by an action on neurogenesis promotion, angiogenesis and synaptic plasticity (Chen et al., 2003, 2005c; Chen and Chopp, 2006). Besides a possible beneficial effect due to proinflammatory reduction and eNOS activation in stroke model, statin treatments increase the secretion of beneficial various growth factors such as vascular endothelial growth factor (VEGF), brain derived neurotrophic factor (BDNF) and tissue plasminogen activator (tPA) (Chen et al., 2005c). In an *in-vitro* model, statins protected cortical neurons from excitotoxicity and increase the neurite outgrowth and synaptogenesis (Zacco et al., 2003; Pooler et al., 2006). Several studies have recently shown the capacity of statins to promote neurogenesis and to improve spatial learning after traumatic brain injury of the dentate gyrus (C3) in the hippocampus (Robin et al., 2014). Although the mechanism leading to neurorestoration is not fully understood, PI3K/Akt activation and prenylation inhibition of signalling molecules such as the RhoA and neurotrophic factor synthesis may be responsible of these improvements (Zheng and Chen, 2007; Wu et al.,

2008; Lu et al., 2007). These preclinical data of statin treatment in stroke model are in line with the epidemiological studies. Statins diminish the severity and apparition of stroke but also help to recover after an acute ischemic stroke (Choi et al., 2015a).

Neurogenesis and growth of neurites induced by statin treatments have been also observed in *in-vitro* using different cell lines. Neuronal cells treated with statins showed an enhancement of neurite sprouting in SH-SY5Y cells (Hughes et al., 2010; Raina et al., 2013), PC12 (Fernández-Hernando et al., 2005), neuro2A (Evangelopoulos et al., 2009; Watanabe et al., 2012), B35 rat neuroblastoma cells (Samuel et al., 2014) and primary culture s(Pooler et al., 2006; Jin et al., 2012).

Moreover, mevalonate, GPP and FPP blocks the neurite growth induced by atorvastatin and pravastatin in neuro-2A and PC12 cells (Fernández-Hernando et al., 2005; Evangelopoulos et al., 2009; Watanabe et al., 2012). All these substrates are the upstream activators of RhoA pathway and ROCK, which leads to neurite stabilization. Statins, but not GPP, reduces the activated form RhoA-GTP and cofilin in hippocampal primary neuron culture, PC12 and B35 cells (Schulz et al., 2004; Fernández-Hernando et al., 2005; Pooler et al., 2006; Samuel et al., 2014). Interestingly a recent study using various cholesterol-blocker agents such as cholesterol scavenger (mβCD), inhibitor of cholesterol transport (U18666A), cholesterol intercalater (α-lysophosphatidylcholine) and cholesterol binding probe (filipinIII) have suggested that cholesterol is necessary for neurite growth (Raina et al., 2013). In addition, atorvastatin-induced neurite growth in cortical primary culture can be reduced by the PIK3 inhibitor (LY294002) and downstream inhibitors of PI3K pathway such as MEK (U0126 and PD98059), mTOR (rapamycin), Akt (triciribine) and GSK3β (SB415286) inhibitors (Jin et al., 2012a).

All these data suggest that modulation of RhoA and PI3K/Akt pathways by statins may be involved in the growth and stabilization of neurites. Although there is no consensus, these complex modulations suggest a potential disease-modifying therapies for PD involving neuroprotection and neurorestoration mechanisms (Figure 14) (Paul et al., 2015; Roy and Pahan, 2011; Reiss and Wirkowski, 2007; Ramirez et al., 2011).

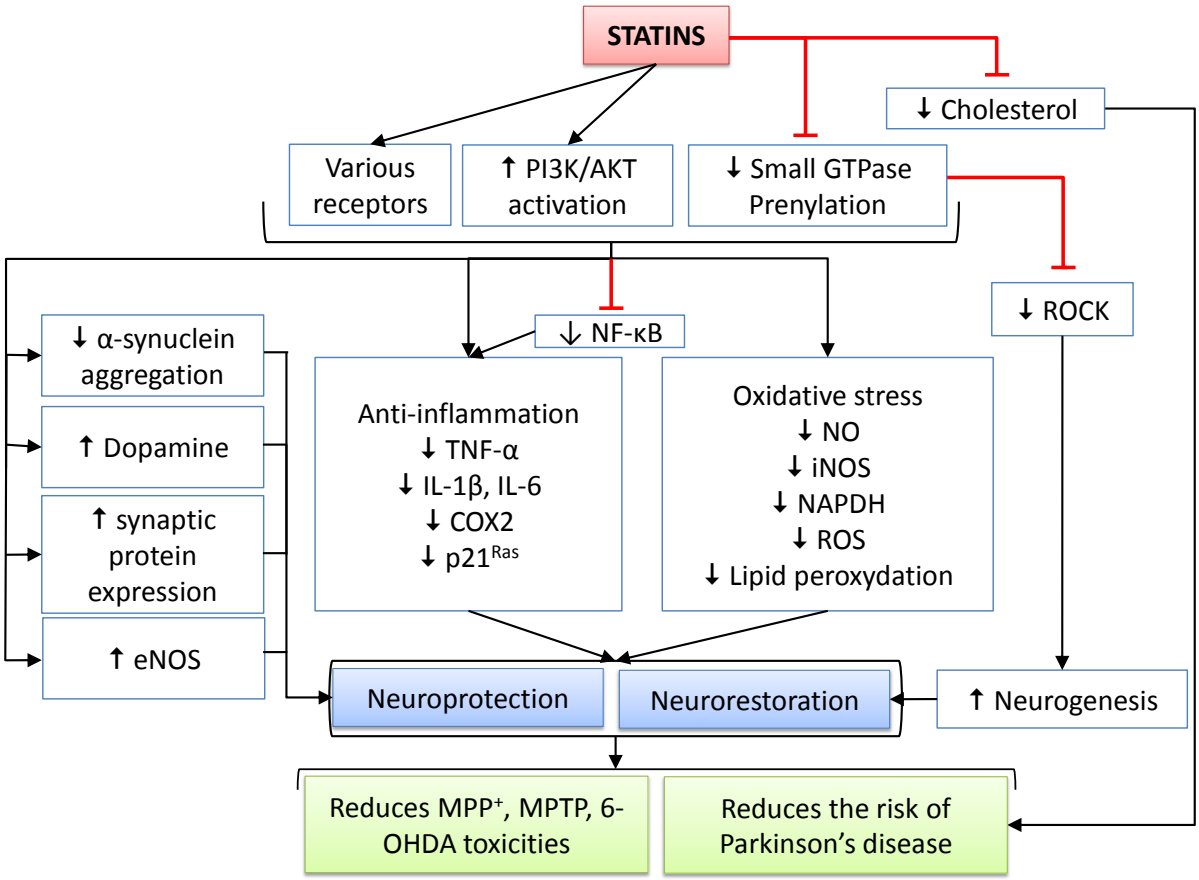


Figure 14. **Hypothetical schematic overview of some pleiotropic mechanisms depicting the neuroprotection of statins in Parkinson's disease.**

COX2, cyclooxygenase 2; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; IL, interleulin; NF-κB, nuclear factor-kappa B; NO, nitric oxide ; $P21^{ras}$, proto-oncogene Proteins p21(ras); PI3K, phosphoinositide 3-kinase; RhoA, ras homolog gene family, member A; ROCK, rho-associated protein kinase; TNF, tumor necrosis factor;

# Principal objectives

The treatment of motors symptoms in PD has been successfully achieved by the therapies addressing the substitution of dopamine deficits such as L-Dopa and dopamine receptor agonists. However, the progressive neurodegeneration taking place during the course of the disease as well as side effects of drug treatments represent a major unmet medical need. Last decade a major effort has been focused in finding a treatment to slow down neurodegenerative mechanisms or to restore lost neuronal systems. Unfortunately, no disease-modifying treatment is available for PD patients so far. Recent epidemiological and preclinical studies on statins have highlighted a potential neuroprotective effect in PD, however the understanding of the neuroprotective mechanism/s triggered by these molecules remain undisclosed.

Although a few studies have demonstrated a modulation of the dopaminergic system and synaptic markers, there is no result on a neurorestorative component of the statin as proposed for other neurological disease (e.g. stroke, spinal cord injury). The cholesterol metabolism is the primary action of statin, however several downstream pathways have been recently identified and suggest a complex action on mechanism involved in neuroprotection and neuroregeneration. It has been found that statins impact biological pathways (e.g. RhoA and PI3K/Akt) controlling the formation and integrity of cytoskeleton and the induction neurite growth. Moreover, statins affect the cholesterol-dependent SREBP-1 pathway that controls gene transcription. While above studies indicate that statins may be involved in neuroregeneration, the specificity of these actions as well as their role in controlling neuronal phenotype remains poorly understood. Few studies have demonstrated a statin-induced modulation of the dopaminergic system, however the impact in the expression of presynaptic dopaminergic markers and their function remain rather unknown. This knowledge represents an important element of validation of the therapeutic potential of statin for the treatment of PD.

In this context, present project aimed to investigate the effects of statins in the presynaptic dopaminergic system as well as the downstream pathways potentially involved in neuroprotection and neuroregeneration. The objective of the studies was also to determine the potential functional impact of statins in the dopamine transport system, a key element of the neuropathological process-taking place in PD. Using SH-SY5Y neuroblastoma cells, a

neuronal cell line with a mild dopaminergic phenotype, we have investigated the statin effects in the expression of presynaptic dopaminergic markers, the genesis of synaptic elements, the functional changes of dopamine transport.

1. Study of statin-induced neurite growth, identification of downstream pathways and potential specific effects compared to other modulators of this neuroregenerative mechanism including RhoA- and PI3K/Akt-pathway inhibitors.
2. Determination of the effect of statins on the phenotypic differentiation towards the dopaminergic pathways. The study aimed to analyse the protein levels and gene transcriptions of dopaminergic presynaptic markers including DAT, VMAT2, SYNGR3, SV2A, SV2C and TH.
3. Investigation of the impact of statin treatments in the functionality of the cellular dopamine transport system with particular focus on the changes in dopamine transport capacity associated to DAT and VMAT2 activities.
4. Study of statin-induced modulation of SREBP-1 in SH-SY5Y neuroblastoma cells. This part aimed to determine the potential role of this cholesterol-dependent pathway in the actions of statins in the dopaminergic system of the SH-SY5Y cells.

Overall, present project aimed to contribute to the validation of the therapeutic potential of statins for the treatment of PD by investigating cellular and molecular effects of these compounds and the downstream mechanism modulating the dopaminergic presynaptic system.

# Results and discussion

# Results and discussion

## IV. Harnessing the trophic and modulatory potential of statins in a dopaminergic cell line

### IV. 1. Abstract

The identification of an effective disease-modifying treatment for the neurodegenerative progression in Parkinson's disease (PD) remains a major challenge. Epidemiological studies have reported that intake of statins, cholesterol lowering drugs, could be associated to a reduced risk of developing PD. *In-vivo* studies suggest that statins may reduce the severity of dopaminergic neurodegeneration. We have investigated the trophic potential of statins and their impact on the expression of dopaminergic synaptic markers and dopamine (DA) transport function in SH-SY5Y cells. Our findings show that statin treatment induces neurite outgrowth involving a specific effect on the complexity of the neurite branching pattern. Statins increased the levels of presynaptic dopaminergic biomarkers such as vesicular monoamine transporter 2 (VMAT2), synaptic vesicle glycoproteins 2A and 2C (SV2C) and synaptogyrin-3 (SYNGR3). Gene expression analysis confirmed a rapid statin-induced upregulation of VMAT2-, SV2C- and SYNGR3-mRNA levels. Assessment of [$^3$H]DA transport in statin-treated cells showed a reduction in DA uptake concomitant to a modification of VMAT2 pharmacological properties. We also observed a nuclear translocation of the sterol regulatory element-binding protein 1 (SREBP-1). Our results suggest that statins induce phenotypic changes in dopaminergic cells characterized by an increase in growth, complexity of structural synaptic elements and expression of key presynaptic proteins with functional impact on the DA transport capacity. Statin-induced changes are likely the result of a downstream modulation of SREBP-1 pathway. Overall, these mechanisms may contribute to the neuroprotective or neurorestorative effects observed in the dopaminergic system and strengthen the therapeutic potential of statins for PD.

# IV. 2. Introduction

Epidemiological studies have identified an association between the use of statins and a diminished risk for developing Parkinson's disease (PD) (Wolozin et al., 2007; Wahner et al., 2008; Gao et al., 2012; Lee et al., 2013) or Alzheimer's disease (Li et al., 2012), thereby suggesting the therapeutic potential of statins in neurodegenerative diseases. The precise molecular mechanism by which statins may decrease the risk of PD is however not yet understood.

Statins might have neuroprotective effects through different molecular pathways. Several studies demonstrated that inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase partially prevented neuronal degeneration *in-vitro* and in animal models of PD. Compelling data showed that statins are able to prevent the neurodegenerative effects induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Ghosh et al., 2009; Castro et al., 2013) and 6-hydroxydopamine (6-OHDA) (Yan et al., 2011a; Xu et al., 2013) treatment and to dampen α-synuclein-induced toxicity (Bar-On et al., 2008; Koob et al., 2010). Statins have been reported to induce restoration of mitochondrial enzyme complex and upregulation of endothelial nitric oxide synthase as well as reduction of proinflammatory responses such as cytokine releases, nuclear factor kappa-B activation and inducible nitric oxide synthase expression (Ghosh et al., 2009; Roy and Pahan, 2011; Kumar et al., 2012; Xu et al., 2013).

In addition to the neuroprotective potential, statins might also stimulate neurorestoration by promoting neurogenesis in rat dentate gyrus (Lu et al., 2007; Robin et al., 2014) and neurite growth in rat cortical and hippocampal primary neuronal cultures (Pooler et al., 2006; Jin et al., 2012) and also in different neuronal cell lines such as PC12 (Sato-Suzuki and Murota, 1996; Schulz et al., 2004), SH-SY5Y (Raina et al., 2013) and neuro-2A (Evangelopoulos et al., 2009; Watanabe et al., 2012). The precise molecular mechanisms for these statin-induced actions remain unclear but it has been suggested that Phosphoinositide 3-kinase (PI3K)/Akt and ras homolog gene family, member A (RhoA) signaling pathways might play a role in statin-induced neurite growth (Schulz et al., 2004; Evangelopoulos et al., 2009; Jin et al., 2012; Raina et al., 2013; Samuel et al., 2014). Inhibition of HMG-CoA reductase by statins leads to inhibition of the mevalonate pathway and cholesterol synthesis. Subsequently, decreasing cholesterol levels will induce the maturation of the transcription

factor sterol regulatory element-binding protein (SREBP) and its nuclear translocation (Kallin et al., 2007; Shao and Espenshade, 2012). Although the transcription activity of SREBP up-regulates expression of multiple genes involved in fatty acid and cholesterol metabolism (Kim and Spiegelman, 1996; Shimano et al., 1999; Shao and Espenshade, 2012), SREBP is also able alter the regulation of expression of neuronal markers (Kallin et al., 2007; Suzuki et al., 2010). For example, ectopic overexpression of SREBP-1 induced a clear mRNA increase in the synaptic vesicles glycoprotein 2A (SV2A) up to 40 folds which correlates to the increase induced with statin treatment in non-neuronal cells (Kallin et al., 2007).

Based on these data, statins have been proposed to be potential disease-modifying drugs for PD (Reiss and Wirkowski, 2007; van der Most et al., 2009; Wang et al., 2011; Malfitano et al., 2014). However, direct or indirect effects of statins on the dopaminergic system, the most affected neurotransmitter system in PD (Lotharius and Brundin, 2002), remain poorly characterized. In this context, we have investigated *in-vitro* effects of statins on growth, phenotypic changes and the impact on dopamine (DA) transporter system in dopaminergic SH-SY5Y cells.

# IV. 3. Materials and methods

## IV. 3.1. Materials

SH-SY5Y (CRL-2266) and BE(2)-M17 (95011816) cells were obtained from ATCC (American Type Culture Collection, Molsheim, France) and ECACC (European Collection of Cell Cultures, Salisbury, UK), respectively. Reagents for cell cultures and Hank's balanced salt solution (HBSS) were purchased to Lonza (Verviers, Belgium). Coating reagent poly-D-lysine and collagen were from Sigma-Aldrich (Diegem, Belgium) and Corning (Lasne, Belgium) respectively. Primary antibodies were βIII-tubulin (MMS-435P or PRB-435P) from Covance (Rotterdam, Netherlands), tyrosine hydroxylase (TH; AB152) from Millipore (Overijse, Belgium), synaptogyrin-3 (SYNGR3; sc-271046) from Santa Cruz Biotechnology (Dallas, TX, USA), SREBP-1 (SAB4502850), dopamine transporter (DAT; D6944) and β-actin (A3853 and A2066) from Sigma-Aldrich, SV2A (119 002), synaptic vesicle glycoprotein 2C (SV2C; 119 202) and vesicular monoamine transporter 2 (VMAT2; 138 302) from Synaptic Systems (Goettingen, Germany). Secondary antibodies were from Life Technologies (Alexa Fluor 488- and 647-conjugated goat anti-mouse and/or anti-rabbit IgG; Gent, Belgium) or LI-COR (IR-dye 680RD or with 800CW donkey anti-mouse and/or anti-rabbit; Leusden, Netherlands) for immunocytochemistry and western blotting, respectively. DAPI, Lipofectamine 2000, high capacity cDNA reverse transcription kit with MultiScribe MuLV transcriptase, Luminaris probe qPCR master mix (low ROX), TaqMan PCR probe (FAM-MGB), Pierce bicinchoninic acid (BCA) protein assay, reagent used for protein electrophoresis and serum for cell cultures were from Life Technologies. CellLytic-M, DA, L-ascorbic acid, pargyline, reserpine were purchased to Sigma-Aldrich. Atorvastatin, GBR-12935, fluvastatin, lovastatin, LY-294,002, pravastatin, simvastatin, tetrabenazine, Y-27632, wortmannin were purchased to Tocris (Abingdon, UK). Microscint-20 and dihydroxyphenylethylamine,3,4-[ring-2,5,6-3H] ([$^3$H]DA, NET673; 34.8 Ci/mmol) were from Perkin Elmer (Zaventem, Belgium).

## IV. 3.2. Cell cultures and drug treatments

SH-SY5Y and BE(2)-M17 neuroblastoma cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) of FBS and maintained in a humidified incubator with 95% air and 5% CO2 at 37 °C. For immunocytochemistry, the cells were seeded (15 x $10^3$) 24 h before the studies on poly-D-lysine and collagen mix pre-coated 96-well plates. The seeding cell density for DA functional transport was 50 x $10^3$ and 17 x $10^3$

cells per well for SH-SY5Y and BE(2)-M17 cells respectively. For gene expression studies, SH-SY5Y cells were plated in 48-well plates at 160 x $10^3$ cells per well for gene expression and in 6-well plates at 1.5 x $10^6$ for western blot analysis. The cells were stimulated with pharmacological agents by incubation (24 h and 48 h) alone or in combination with single or multiple increasing doses.

### IV. 3.3. Immunocytochemistry and High-content image analysis

The effects of pharmacological treatments on protein expression and neurite growth were analysed by immunofluorescence method using antibodies against the synaptic markers. Briefly, cells were washed with PBS followed by fixation with 4% paraformaldehyde for 30 min (room temperature) and permeabilization (triton X-100 0.05% - 10 min). The non-specific binding was blocked with blocking solution (3% of BSA and 5% of normal goat serum in PBS) for 1 h. The cells were incubated overnight at 4 °C with primary antibodies against DAT (1:500), SREBP-1 (1:500), SV2A (1:500), SV2C (1:500), SYNGR-3 (1:100), TH (1:500), VMAT2 (1:500) or βIII-tubulin (1:3000) in blocking solution. After washing (4 x 5 min), the cells were incubated with species-specific corresponding secondary antibodies labelled with Alexa Fluor 488 or 647 in blocking solution. Nuclear counterstaining was performed by incubation with DAPI and by further washing steps (5 x 5 min).

Immunofluorescence signal was analysed using a high-content imaging microscopy system (BD Pathway-855 Bioimager System using BD Attovision software, Becton-Dickinson, Erembodegem, Belgium) in PBS medium. Series (2 by 3) of images were acquired in 3 non-superposing image fields per well using a 20x objective (0.75NA, Olympus, Berchem, Belgium). The neurite length, the fluorescent intensity of neuronal markers and nuclear translocation were analysed using a specific image analysis algorithms developed using Cellenger software package (Definiens, München, Germany). Neurite growth was determined using parameters of neurite length and number previously classified as primary or secondary neurite. Number of nodes was also used as surrogate parameter of neurite branching. Cytoplasmic and nuclear intensity parameters were used for determination of protein levels and nuclear translocation. Both quantifications use the cell compartmentalization defined by segmentation of the nucleus and cytoplasm and nucleus using DAPI and βIII-tubulin staining respectively. For quantification of protein levels, the relative intensity of cytoplasm was measured and the background value was subtracted to the

results. The nuclear translocation was measured by quantification of the nuclear/cytoplasmic intensity ratio.

### IV. 3.4. Western blot analysis

Cells were rinsed with PBS and lysed by 30 min incubation with CellLytic-M containing cOmplete protease inhibitor cocktail (Roche Diagnostics, Vilvoorde, Belgium) at 4 °C. The cell lysates were centrifuged at 15,000 g for 20 min at 4 °C and supernatants were used for western blotting. Protein levels were determined with BCA kit and electrophoresis was performed using 30 µg of denatured (95 °C, 10 min) cell lysates in loading buffer (62.5 mM Tris-HCl pH 6.8, 2 mM EDTA, 10% glycerol, 2% SDS, 0.002% bromophenol blue, 100 mM DTT). Proteins were separated (2 h, 140V) in 4 to 12% SDS-Polyacrylamide gradient gel in NuPAGE MES SDS running buffer. Proteins were transferred onto PVDF membranes (Millipore, 0.45 µm) at 25V for 30 min in NuPAGE transfer buffer. PDVF-membranes were saturated (1 h) in LI-COR blocking buffer and incubated overnight (4°C) with primary antibodies in blocking buffer: SV2A (1:1,000), SV2C (1:1,000), DAT (1:1,000), SYNGR3 (1:200), VMAT2 (1:1,000) and β-actin (1:5,000). After washing (3 x 15 min) with PBS-tween 0.1% (PBST), the membranes were incubated (1 h) with secondary antibodies in blocking buffer. Protein bands were analysed after washing steps (3 x 15 min, PBST) by densitometry using a LI-COR Odyssey CLX scanner. Relative protein levels were calculated after normalization to β-actin levels in the same sample.

### IV. 3.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total cellular RNA was isolated using the RNeasy mini kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. Briefly, cDNA was synthetized from 1.5 µg of total RNA using high capacity cDNA reverse transcription kit (20 µl). The quantitative real-time PCR experiments were performed using specific TaqMan gene expression probes for SV2A (Hs00372069_m1), SV2C (Hs00392676_m1), SREBP1 (SREBF1; Hs01088691_m1), SREBP2 (SREBF2; Hs01081784_m1), SYNGR3 (Hs00188379_m1), VMAT2 (SLC18A2; Hs00996835_m1), TH (Hs00165941_m1), DAT (SLC6A3; Hs00997364_m1) and β-actin (ACTB; Hs99999903_m1). The reactions were performed in ViiA 7 RT-PCR system (Applied Biosystems) using 25 ng of cDNA sample and the recommended concentration of the specific probe and qPCR master mix Luminaris. PCR

reactions were run in triplicate and the fold-changes in mRNA levels were calculated using $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) and normalized to β-actin mRNA levels.

### IV. 3.6. Dopamine transport assay

DA transport assay was determined according to Janowsky et al. protocol (Janowsky et al., 2001) with minor modifications. Briefly, the cell culture medium was replaced by HBSS (pH 7.4) containing 10 mM HEPES, 10 µM pargyline and 1 mM L-ascorbic acid. The transport was initiated by adding 12.5 nM [$^3$H]DA in incubation buffer. Cells were incubated for 15 or 60 minutes in transport buffer and DA uptake was stopped by adding 300 µl of cold HBSS at 4°C. Buffer was quickly removed by aspiration. The incorporated radioactivity was determined by adding Microscint-20 scintillation liquid (50µl) and reading using TopCount NXT microplate luminescence and scintillation counter (Perkin Elmer). For transport inhibition experiments, cells were pre-incubated (30 min) with selected compound GBR-12935, reserpine or tetrabenazine. For kinetic studies, cells were incubated with increasing concentrations (0.1-3 µM) of DA in presence of 50 nM of [$^3$H]DA. Non-specific DA uptake was subtracted to the results and determined by incubation of cells with radioligand in the presence of 30 µM GBR-12935. The total protein concentration was measured using the BCA kit.

### IV. 3.7. VMAT2 transient transfections

Human VMAT2 cDNA was subcloned into the expression vector pcDNA3.1(+) (Invitrogen, Gent, Belgium) by double digestion of HindIII and KpnI enzymes by Genscript (Piscataway, NJ, USA). For inducing VMAT2 overexpression, cells were transfected with the plasmid 1.25 µg/ml of pcDNA3.1-VMAT2-Hu prepared in Lipofectamine 2000 (2 µg/ml). Plasmid solution and Lipofectamine 2000 were pre-diluted separately for 5 min in serum free medium and mixed for 30 min before to be added into the well. Cells were used 72 h after transfection.

### IV. 3.8. Data analysis

Results are presented as means ± S.E.M. from a minimum of three independent experiments in duplicate or triplicate unless otherwise stated. Data were analysed with GraphPad Prism software (La Jolla, CA, USA) using either Student's t-test or ANOVA with

Bonferroni's post hoc test. $P<0.05$ was considered significant to assess the difference between conditions. Half maximal effective concentration ($EC_{50}$) or inhibitory concentration ($IC_{50}$), maximal effect ($E_{max}$) or inhibition effect ($I_{max}$), Hill coefficient ($n_H$) and basal level pharmacological parameters were generated with nonlinear regression and fitted to four-parameter logistic curve (4PL);

$$Y = basal\ level + \frac{maximal\ effect - basal\ level}{1 + 10^{(LogEC_{50}\ or\ IC_{50} - X)n_H}}$$

and normalized equation;

$$Y = \frac{100}{1 + 10^{(LogEC_{50}\ or\ IC_{50} - X)n_H}}$$

The Michealis-Menten kinetics equation was applied to calculate the DA transport constants kinetics for maximal rate ($V_{max}$) and substrate affinity ($k_m$);

$$Velocity\ = \frac{V_{max}\ [Dopamine]}{K_m + [Dopamine]}$$

When necessary, fitting analysis for a 2-components curve was evaluated using a Fisher-test (F-test) for comparison between one-site and two-site nonlinear regression (high and low affinity sites). Pharmacological parameters were compared using extra sum-of-squares F-test analysis.

# IV. 4. Results

## IV. 4.1. Statin-induced neurite growth in SH-SY5Y neuroblastoma cells

Trophic activity of statins on total neurite growth has been previously reported (Evangelopoulos et al., 2009; Jin et al., 2012; Raina et al., 2013; Samuel et al., 2014). However, their potential impact on the complexity of neuritic structures and the specificity versus the other neurotrophic compounds remains unclear. Using βIII-tubulin immunocytochemistry (Fig. 1a and b), we studied the impact of statins on both the neuritic growth and branching in SH-SY5Y cells. High-content image analysis demonstrated a significant neurite growth induced by lovastatin (Fig. 1c and g) and simvastatin (Fig. 1d and g) after 24 h of incubation. The statin treatment also induced a significant increase in the number of neurite branching nodes (Fig. 1h). Atorvastatin and fluvastatin also induced a similar increase in neurite length (Fig. 1g). An own designed image analysis algorithm allowing the detection of primary and secondary neurites revealed that the lovastatin- and simvastatin-induced neurite growth affected both the length (Fig. 1k) and the number (Fig. 1m) of secondary neurites. No significant changes were found for the length (Fig. 1j) or the number (Fig. 1l) of primary neurites. These data confirm the trophic effect of statins and demonstrate that they specifically affect the genesis of secondary neurites, which increases the complexity of the neuritic network.

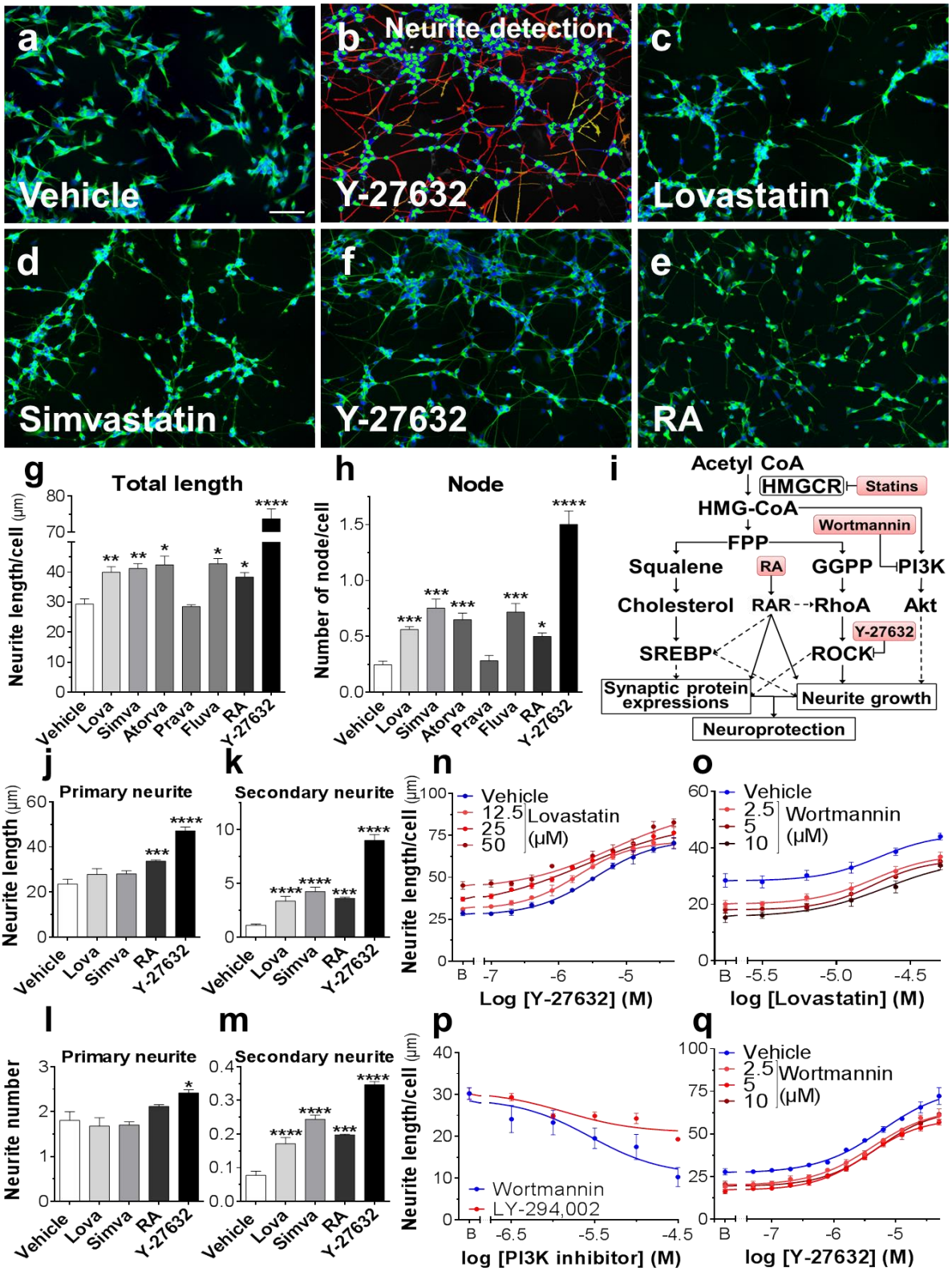


Figure 15. (Figure 1.) **Statins-induced neurite outgrowth with a complex branching independent to the PI3K and RhoA downstream mevalonate pathway in SH-SY5Y cells after 24 h treatment**

(**a**) Representative image of βIII-tubulin (green) and DAPI (blue) staining in SH-SY5Y cells. b, segmentation mask comprising the layers of nucleus (green), cytoplasm (blue), primary (red) and

secondary (orange) neurite. (**b-e**) Treated cells with lovastatin (Lova) 30 µM, simvastatin (Simva) 30 µM, Y-27632 50 µM and retinoic acid (RA) 10 µM. (**g-h**) quantification of neurite length (**g**, $F_{(9,48)}$=34.18, *P*<0.0001) and nodes in neurite branching (**h**, $F_{(9,47)}$=37.80, *P*<0.0001) per cell with primary neurite treated with RA 10 µM, Y-27632 50 µM and statins 30µM for lovastatin, simvastatin, atorvastatin (Atorva), pravastatin (Prava) and fluvastatin (Fluva). (**i**) mevalonate pathway and pharmacological tools used to study it (HMGCR, HMG-CoA reductase; GGPP, geranylgeranyl-pyrophosphate; FPP, farnesyl-pyrophosphate). (**j-k**) primary (**j**: $F_{(6,58)}$=13, *P*<0.0001) and secondary (**k**, $F_{(6,52)}$=42.60, *P*<0.0001) neurite length/cell. (**l-m**), primary neurite/cell (**l**, $F_{(6,59)}$=3.21, *P*=0.0085) and secondary neurite number/primary neurite (**m**, $F_{(6,50)}$=36.82, *P*<0.0001). (**n-q**) dose-responses effects of compounds alone and in combination on neurite length; (**n**) Y-27632 combined with lovastatin; o, lovastatin combined with wortmannin; (**p**) wortmannin and LY-294,002; (**q**) Y-27632 combined with wortmannin. Results represent means ± S.E.M. (n=3-12). (**n-q)** results were fitted by nonlinear regression with a 4PL equation. Statistical analyses were performed by one-way ANOVA followed by Bonferroni post hoc test; **P*<0.05, ***P*<0.01, ***P*<0.001, *****P*<0.0001, vs corresponding vehicle treated cells. B, basal level. Scale bar indicates 50 µm.

## IV. 4.2. Differential trophic effects of statins in SH-SY5Y and potential downstream pathways

Neurite growth induced by HMG-CoA reductase inhibition may result from various downstream cellular mechanisms of mevalonate pathway including the RhoA and PI3K pathways (Fig. 1i). These pathways regulate the polymerization of actin filaments involved in the neurite sprouting and have been proposed to contribute to statins trophic properties by inhibition of PI3K and Rho-associated protein kinase (ROCK, a downstream effector of RhoA) (Fig. 1i) (Schulz et al., 2004; Evangelopoulos et al., 2009; Racchetti et al., 2010; Jin et al., 2012; Raina et al., 2013). In this context, we examined the effects of ROCK and PI3K inhibitors on statin-induced neurite growth.

Inhibition of ROCK by treatment with Y-27632 produced an increase in total neurite length (Fig. 1f and g). Nonlinear regression analysis revealed an $EC_{50}$ of 3.1±0.23 µM (Fig. 1n). Under identical experimental conditions, lovastatin exhibited a slightly lower potency ($EC_{50}$ of 17.4±2.1 µM) (Fig. 1o). Moreover, treatment with Y-27632 resulted in a significant growth of primary and secondary neurites (Fig. 1j-m). Co-treatment conditions showed that Y-27632 dose-response curve for total length was shifted up by lovastatin in a dose-dependent manner and it was accompanied by a change in the slope of the curve with $n_{H=}$1.12±0.04 for Y-27632 vs 0.86±0.06 for Y27632 + lovastatin 50µM ($F_{(1,62)}$=7.35, *P*=0.0086) (Fig. 1n). Similar effects were observed for primary and secondary neurite length and number (Y-27632 vs Y-27632 + lovastatin 50 µM: primary length, $n_{H=}$1.3±0.09 vs 0.90±0.07, $F_{(1,140)}$=11.53, *P*=0.0009; secondary length, $n_H$=1.26±0.09 vs 1.00±0.08, $F_{(1,140)}$=4.49, *P*=0.0358; primary number, $n_H$=1.48±0.24 vs 0.84±0.16, $F_{(1,140)}$=4.57, *P*=0.0342; secondary number: $n_H$=1.32±0.09 vs 1.01±0.07, $F_{(1,140)}$=6.42, *P*=0.0123) (Fig. 2a, d, g and j). However, no

changes were observed for the maximal response over baseline between the combined treatment (Y-27632 + lovastatin) and the Y-27632 alone (Y-27632 vs Y-27632 + lovastatin 50 µM: total length, $E_{max}$=44.1±1.2 vs 46.2±2.4 µm, $F_{(1,64)}$=0.21, *P*=0.6474; primary length, $E_{max}$=28.5±0.7 vs 24.6±2.3 µm, $F_{(1.136)}$=1.5, *P*=0.2214; secondary length, $E_{max}$=11.8±0.7 vs 13.7±1.5 µm, $F_{(1.136)}$=2.01, *P*=0.1584; primary number, $E_{max}$=1.03±0.03 vs 0.54±0.03, reduction $F_{(1.140)}$=94.65, *P*<0.0001; secondary number, $E_{max}$=0.35±0.02 vs 0.36±0.03, $F_{(1.136)}$=0.01, *P*=0.9031) (Fig. 1n, 2a, d, g and j). These observations likely suggest a RhoA-independent trophic effect of lovastatin.

The treatment of SH-SY5Y cells with PI3K inhibitors induced a significant dose-dependent neurite retraction with an $I_{max}$ of 67.2±4.7% and 37.7±1.7% and $IC_{50}$ of 4.4±0.6 µM and 2.8±1.2 µM for wortmannin and LY-294,002 respectively (Fig. 1p). This reduction impacted both the number and length of primary and secondary neurites (one-way ANOVA, vehicle vs wortmannin, primary neurite length: $F_{(3,44)}$=46.37, *P*<0.0001 and number: $F_{(3,44)}$=25.77, *P*<0.0001; secondary neurite length: $F_{(3,44)}$=13.87, *P*<0.0001 and number: $F_{(3,44)}$=5.2, *P*=0.0036) (Fig. 2b, c, e, f, h, k and l). In order to determine the potential downstream role of PI3K pathway in the HGM-CoA- and ROCK-inhibition-mediated effects, we co-treated SH-SY5Y cells with lovastatin or Y-27632 and wortmannin. Both lovastatin- and Y-27632-induced neurite growth response curve were shifted down in presence of wortmannin and in a dose-dependently manner (Fig. 1o and q). In both cases, the absolute responses over baseline of the trophic compounds remained unchanged which suggests that PI3K pathway is not involved in statin- or ROCK-inhibition mediated regulation (lovastatin vs lovastatin + wortmannin 10µM: $E_{max}$=16.5±1.3 vs 17.6±0.52 µm, $F_{(1,33)}$=0.70, *P*=0.4066; Y-27632 vs Y-27632 + wortmannin 10 µM: $E_{max}$=50.1±4.4 vs 44.3±1.7 µm, $F_{(1,70)}$=1.88, *P*=0.1751) (Fig. 1o and q). However, lovastatin inhibited the retraction of primary neurite length and number induced by wortmannin with an $E_{max}$ of 11.59±1.4 vs 17.6±1.4 µm ($F_{(1,76)}$=5.23, *P*=0.025) and 0.088±0.058 vs 0.642±0.051 primary neurite/cell ($F_{(1,80)}$=47.39, *P*<0.0001) for lovastatin and lovastatin + wortmannin 10 µM respectively (Fig. 2b and h). In contrast, Y-27632 did not revert the absolute wortmannin-induced retraction of primary neurite ($E_{max}$=31.6±1.5 vs 32.8±1.3 µm, $F_{(1,136)}$=0.348, *P*=0.5560; $E_{max}$=0.84±0.068 vs 0.88±0.07 primary neurite/cell, $F_{(1,124)}$=0.14, *P*=0.7094) (Fig. 2c and i).

To identify potential differences with other trophic compounds, we also investigated the effects of retinoic acid (RA), an agonist of RA receptors (RAR) (Fig. 1i) (Constantinescu

et al., 2007; Lopes et al., 2010), which plays a role in the dopaminergic differentiation and the growth of SH-SY5Y cells (Fig. 1e and g). Treatment of SH-SY5Y cells with RA induced specific and stronger effect on the length and the number of secondary neurites but a minor effect on the primary neurite length (Fig. 1k, l and m).

Our data demonstrate that ROCK inhibition and RAR activation induce a trophic effect in SH-SY5Y cells while PI3K inhibition clearly induces neurite retraction. In contrast to statins, ROCK inhibition and RAR activation induce an overall extension of both primary and secondary neurites. Taken together, our results suggest that lovastatin-induced trophic effects are independent of RhoA and PI3K pathways and counteract neurite retraction following PI3K inhibition.

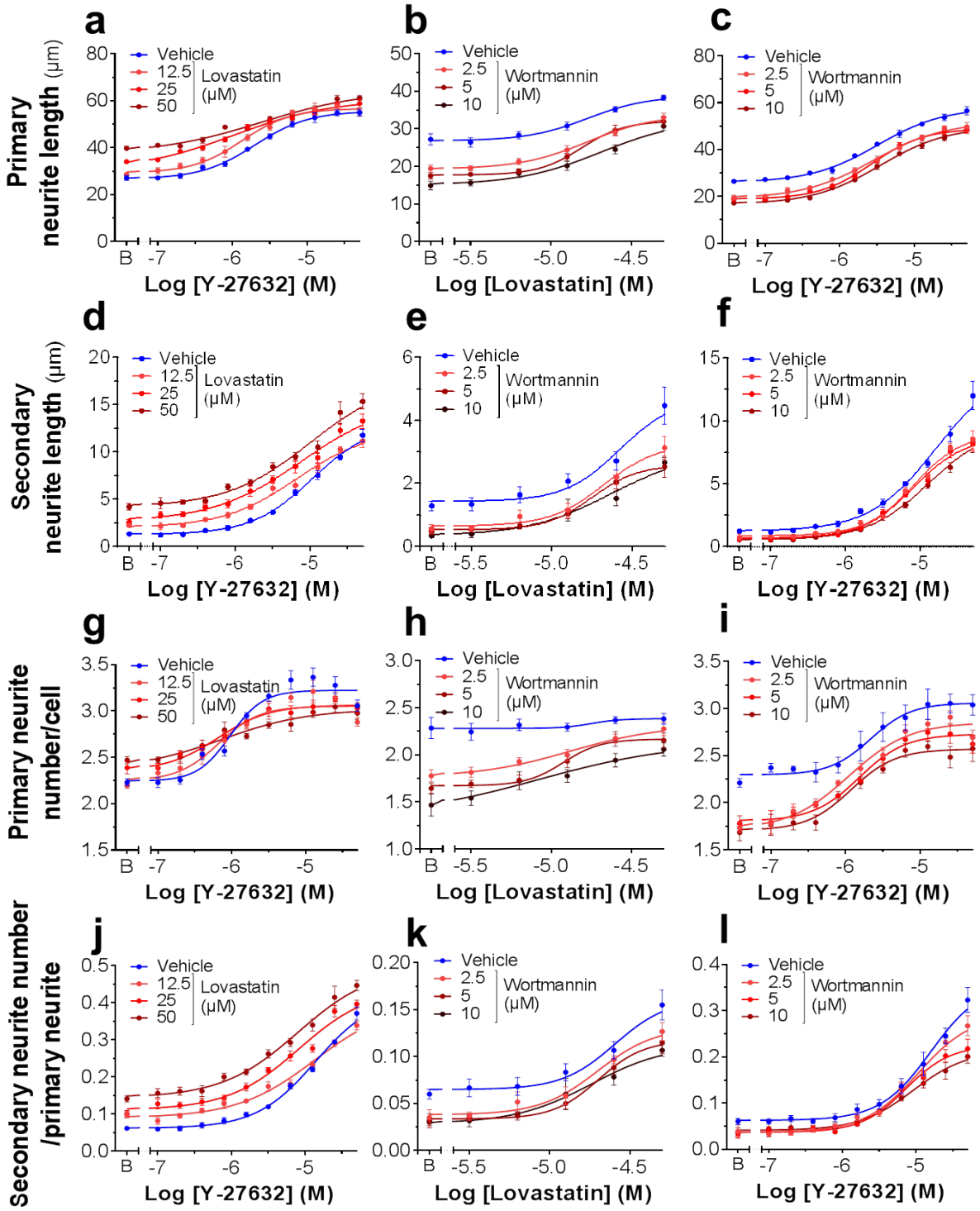


Figure 16. (Figure 2.) **Lovastatin but not ROCK inhibitor reduces neurite retraction induced PI3K inhibitor after 24 h of treatment in SH-SY5Y cells**

Effects of Y-27632 or lovastatin dose-responses alone and in combination with lovastatin or wortmannin treatment on neurite growth complexity. (**a, d, g** and **j**) Y-27632 combined with lovastatin. b, e, h, and k, lovastatin combined with wortmannin. (**c, f, i,** and **l**) Y-27632 combined with wortmannin. Primary neurite length (**a-c**), secondary neurite length (**d-f**), primary neurite number (**g-i**) and secondary neurite number (**j-l**) were analysed for co-treatments. Results represent means ± S.E.M. (n=3) and fitted by nonlinear regression with a 4PL equation. B, basal level.

## IV. 4.3. Statins-induced up-regulation of presynaptic markers

The trophic effects of statins found on neurite growth may trigger phenotypic changes of the synapse. Thus, we investigated the levels of several key proteins of the synapse and particularly of the dopaminergic pre-synaptic system using both immunofluorescence and gene expression analysis. We observed an increase in VMAT2, SV2A, SV2C, TH, SYNGR3 and DAT protein levels in SH-SY5Y cells after treatment with lovastatin or simvastatin (Fig. 3a). High content image analysis demonstrated that both lovastatin and simvastatin induced a dose- and time-dependent increase in synaptic protein levels which reached maximal effect 48 h after treatment (Fig. 3b-g). The maximum increase in fluorescence intensity was observed by lovastatin (30 µM) with a rank order among studied markers such as SYNGR3 > SV2C > SV2A = VMAT2 > TH > DAT (Fig. 3b-g). Only minor differences were noted for simvastatin (SYNGR3 > SV2C = TH > SV2A > VMAT2 > DAT, 30 µM). The potency of the effect was similar for both statins after 24 h with an $EC_{50}$ of 4.12±2.09 µM for lovastatin and 2.07±1.17 µM for simvastatin which remained unchanged at 48 h (Fig. 3b-g). Under the same conditions, the trophic compounds, Y-26732 or RA, did not lead to any significant amplification of fluorescence signal for the studied markers (Fig. 3b-g).

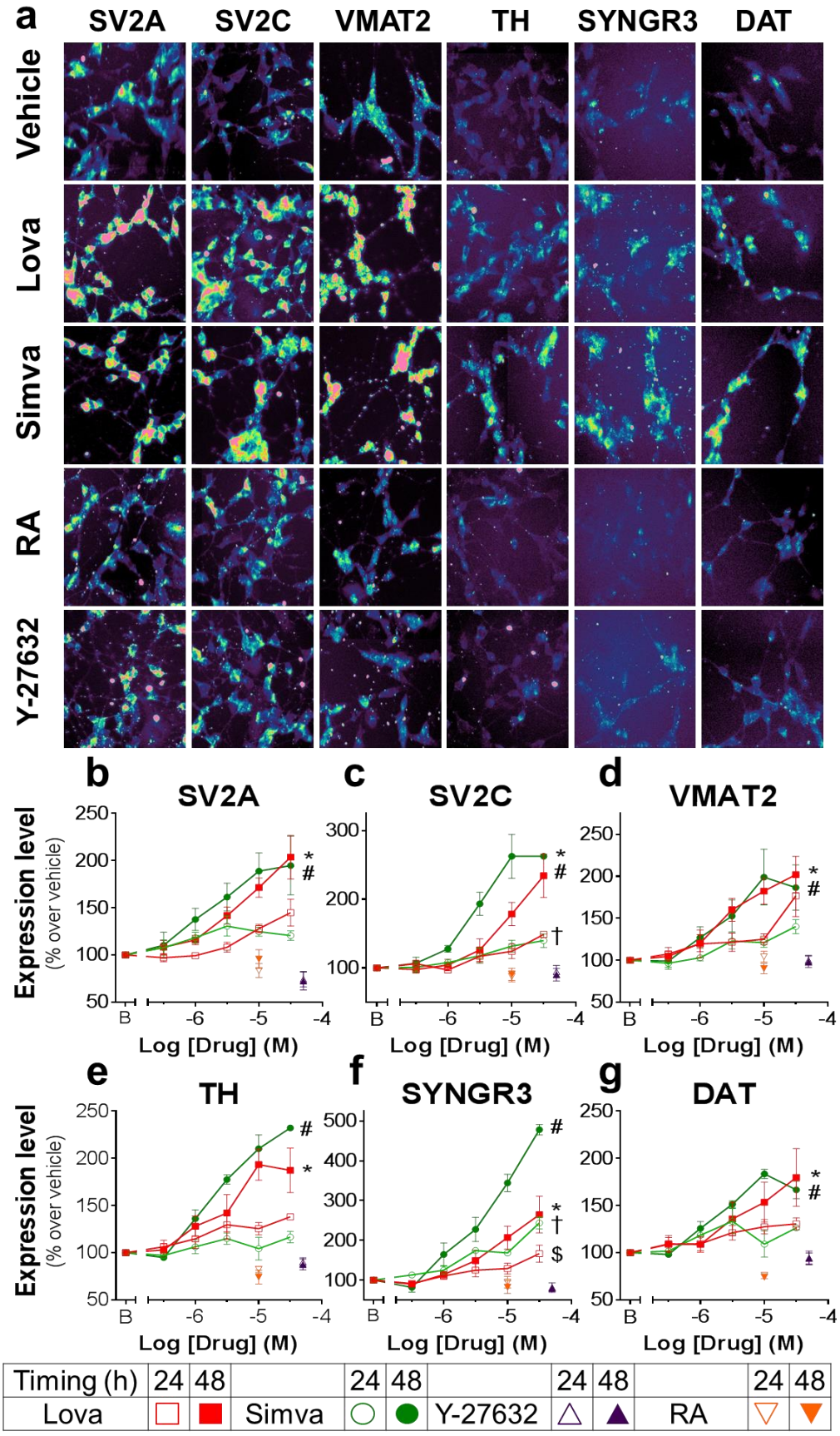


Figure 17. (Figure 3.) **Expression of dopaminergic synaptic markers is enhanced by the statins in SH-SY5Y cells**

(**a)** representative fluorescence images of synaptic proteins SV2A, SV2C, VMAT2, TH, SYNGR3 and DAT after 48 h treatment with lovastatin (Lova) 30 µM, simvastatin (Simva) 30µM, Y-27632 50 µM and retinoic acid (RA) 10 µM. The ratiometric pseudo-color images were generated to enhance the contrast of fluorescence intensity. (**b-g)** quantification of SV2A (**b**), SV2C (**c**), VMAT2 (**d**), TH (**e**), SYNGR3 (**f**) and DAT (**g**) expression levels in cells treated for 24 h or 48 h with lovastatin and simvastatin dose-responses and Y-27632 50 µM and RA 10 µM. Results represent means ± S.E.M. (n=3-6). Statistical analyses were performed by two-way ANOVA (SV2A: treatment effect: $F_{(12,127)}$=7.54, $P$<0.0001; Time: $F_{(1,127)}$=20.72, $P$<0.0001; Interaction: $F_{(33,96)}$=3.1, $P$=0.9991. SV2C: treatment effect: $F_{(12,101)}$=19.84, $P$<0.0001; Time: $F_{(1,101)}$=55.04, $P$<0.0001; Interaction: $F_{(12,101)}$=7, $P$<0.0001. VMAT2: treatment effect: $F_{(12,149)}$=7.67, $P$<0.0001; Time: $F_{(1,149)}$=22.30, $P$<0.0001; Interaction: $F_{(12,149)}$=2.33, $P$=0.009. TH: treatment effect: $F_{(12,86)}$=8.67, $P$<0.0001; Time: $F_{(1,86)}$=32.26, $P$<0.0001; Interaction: $F_{(12,86)}$=3.96, $P$=0.009**.** SYNGR3: treatment effect: $F_{(12,92)}$=28.20, $P$<0.0001; Time: $F_{(1,92)}$=33.71, $P$<0.0001; Interaction: $F_{(12,86)}$=3.92, $P$<0.0001**.** DAT: treatment effect: $F_{(12,88)}$=7.35, $P$<0.0001; Time: $F_{(1,88)}$=11.49, $P$=0.001; Interaction: $F_{(12,88)}$=1.75, $P$=0.0699) followed by Bonferroni post hoc test; */#/$/† were represented when a point of the curve was at least $P$<0.05 vs corresponding baseline for lovastatin 48 h (*), simvastatin 48 h (#), lovastatin 24 h ($), simvastatin 48 h (†) treatments. B, basal level. Scale bar indicates 20 µm.

Immunoblot analysis of cell protein extracts showed that lovastatin induced an increase in VMAT2, SV2C and SYNGR3 protein expression levels with a maximal significant effect at 48 h (Fig. 4a and c-e). No detectable changes were found for SV2A (Fig. 4a and b) and DAT (data not shown). Moreover, no significant changes were observed after treatment with the ROCK inhibitor Y-27632 (Fig. 4).

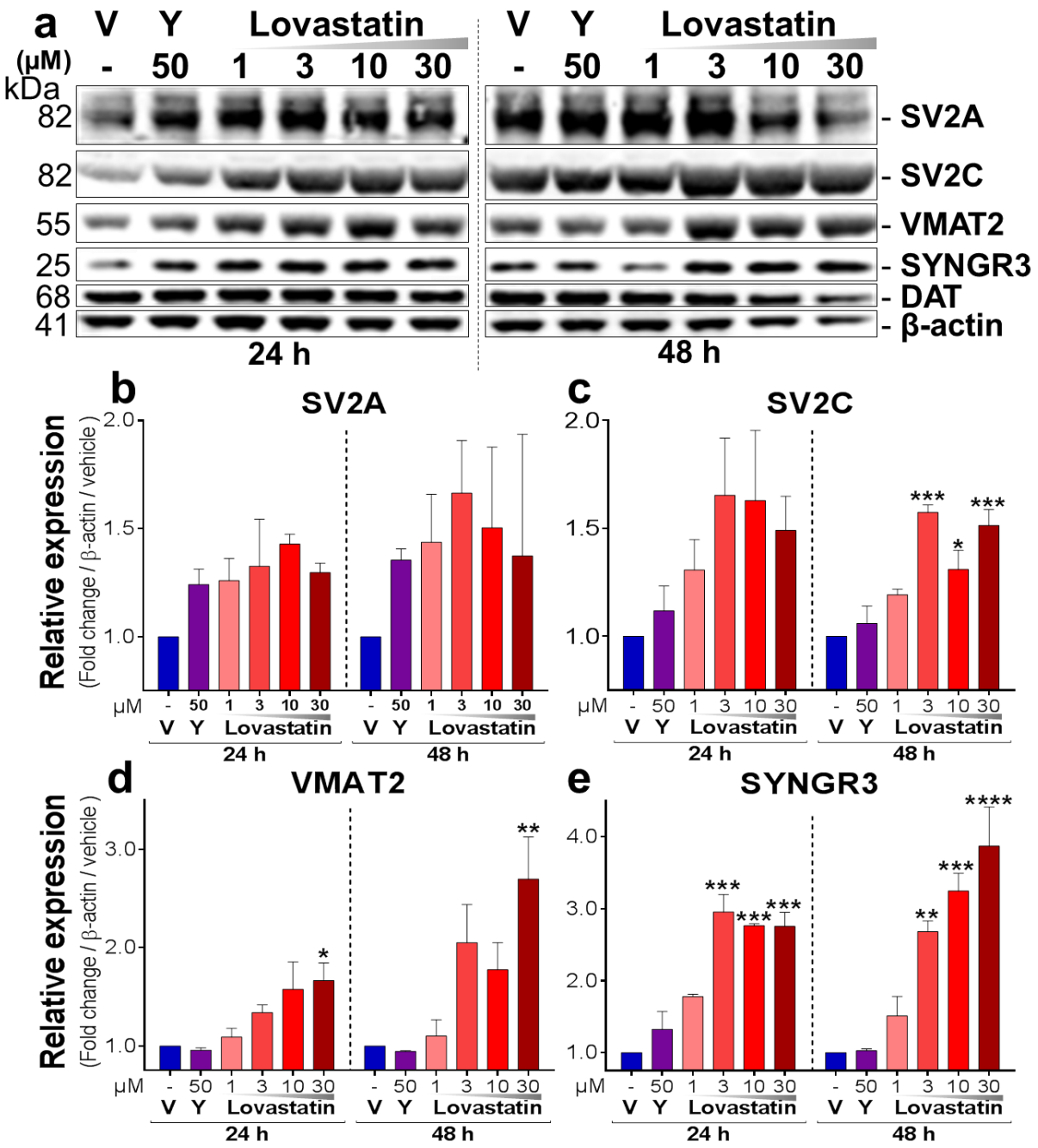


Figure 18. (Figure 4.) **Lovastatin induces an increase in synaptic protein makers related to dopaminergic system in SH-SY5Y cells**

(**a**) representative immunoblots of SV2A (82 kDa), SV2C (82 kDa), VMAT2 (75 kDa), SYNGR3 (25 kDa), DAT (68 kDa) and β-actin (41 kDa) from cells treated 24h and 48 h with the Y-27632 (Y) 50μM and lovastatin dose-response. (**b-e**) Optical density measurements corresponding to SV2A (**b**), SV2C (**c**), VMAT2 (**d**) and SYNGR3 (**e**) immunoblots and normalized over β-actin. Results represent means ± S.E.M. (n=3). Statistical analyses were performed by one-way ANOVA (SV2C 48h: $F(5,12)=15.36$, $P<0.0001$; VMAT2 24 h: $F(5,12)=4.41$, $P=0.0164$; VMAT2 48 h: $F(5,12)=6.83$, $P=0.0031$; SYNGR3 24 h: $F(5,12)=26.30$, $P<0.0001$; SYNGR3 48 h: $F(5,12)=19.76$, $P<0.0001$) followed by Bonferroni post hoc test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, vs corresponding vehicle treated cells. V, vehicle.

We investigated whether statin-induced increase in synaptic proteins was due to an up-regulation of gene transcriptions or to an effect in protein stabilities. Quantitative PCR using mRNA-specific probes showed significantly higher levels of mRNAs coding for the synaptic proteins *SV2A, SV2C, VMAT*2 and *SYNGR3* after 6 h of treatment with lovastatin (Fig. 5a-d). The increased levels of *SV2A* mRNA remained stable at 24 h of incubation (Fig. 5a) while they decreased over time for *SV2C* and *VMAT2* from 6 h to 48 h (Fig. 5b and c). We observed a significant up-regulation of *SYNGR3* mRNA levels at all treatment times (Fig. 5d). No changes in the mRNA levels for *TH* were detected following lovastatin treatment (Fig. 5e). *SREBP-2* mRNA levels, but not those of *SREBP-1*, were enhanced after 12 h of incubation by the statin (Fig. 5f and g). The ROCK inhibitor Y-27632 did not modify mRNA levels of any of the studied synaptic proteins (Fig. 5a-e) RA treatment stimulated mRNA levels of *SREBP-1, TH, SYNGR3* with maximal effects at 12 h, 24 h and 48 h respectively (Fig. 5d-f). A very low level of DAT mRNA was detected which did not allow an accurate quantification in SH-SY5Y cells (data not shown).

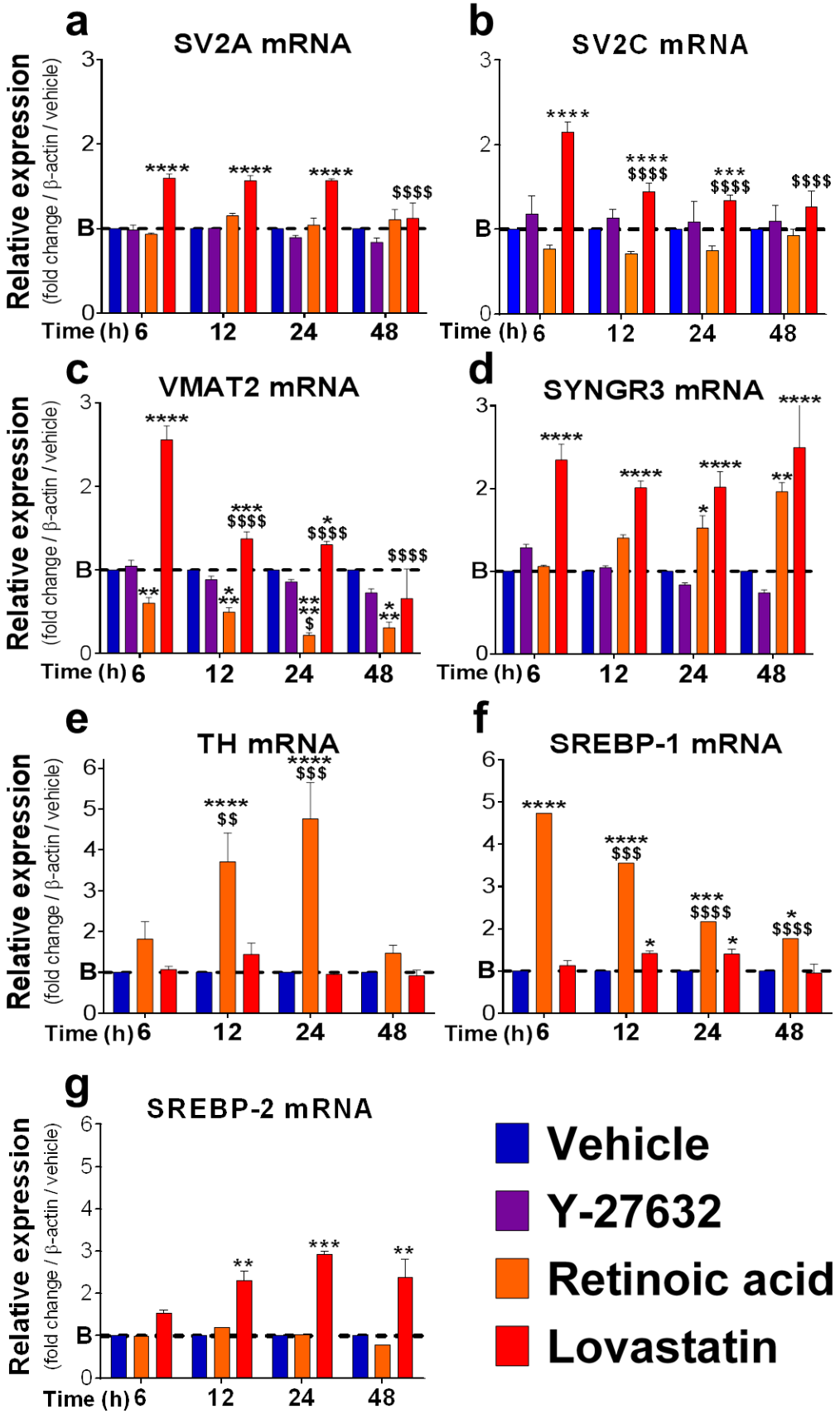


Figure 19. (Figure 5.) **Lovastatin up-regulates mRNA expression levels of dopaminergic synaptic proteins**

(**a-g)**, time-course study (6-48 h) of SV2A (**a**), SV2C (**b**), VMAT2 (**c**), SYNGR3 (**d**), TH (**e**), SREBP-1 (**f**) and SREBP-2 (**g**) mRNA expression normalized over β-actin in SH-SY5Y cells treated with lovastatin 30 µM, Y-27632 50 µM, RA 10 µM. Results represent means ± S.E.M. (n=3). Statistical analyses were performed by two-way ANOVA (SV2A: treatment effect: $F_{(3,53)}$=93.43, *P*<0.0001; Time: $F_{(3,53)}$=7.78, *P*=0.0002; Interaction: $F_{(9,53)}$=6.46, *P*<0.0001. SV2C: treatment effect: $F_{(3,53)}$=49.81, *P*<0.0001; Time: $F_{(3,53)}$=5.426, *P*=0.0025; Interaction: $F_{(9,53)}$=5.28, *P*<0.0001.VMAT2: treatment effect: $F_{(3,47)}$=80.07, *P*<0.0001; Time: $F_{(3,47)}$=29.41, *P*<0.0001; Interaction: $F_{(9,47)}$=13.69, *P*<0.0001. SYNGR3: treatment effect: $F_{(3,52)}$=47.16, *P*<0.0001; Time: $F_{(3,52)}$=1.20, *P*=0.320; Interaction: $F_{(9,52)}$=2.60, *P*=0.0148. TH: treatment effect: $F_{(2,36)}$=35.29, *P*<0.0001; Time: $F_{(3,36)}$=6.64, *P*=0.0011; Interaction: $F_{(3,36)}$=5.91, *P*=0.0002. SREBP-1: treatment effect: $F_{(2,16)}$=42.35, *P*<0.0001; Time: $F_{(3,16)}$=326.6, *P*<0.0001; Interaction: $F_{(3,16)}$=39.43, *P*<0.0001. SREBP-2: treatment effect: $F_{(2,16)}$=40.88, *P*<0.0001; Time: $F_{(1,16)}$=2.23, *P*=0.1240; Interaction: $F_{(6,16)}$=2.21, *P*=0.0964) followed by Bonferroni post hoc test;*/#*P*<0.05, **/##*P*<0.01, ***/###*P*<0.001, ****/####*P*<0.0001, * vs corresponding vehicle treated cells, # vs corresponding 6 h of treatment. B, basal level.

Altogether, these data suggest that increases of SYNGR3, VMAT2, SV2A and SV2C protein levels are rather associated to an increase in transcription of their corresponding genes. In contrast, the increase in protein levels observed for TH and DAT is independent of transcriptional activation. The statin-induced effects are at variance with those observed after treatment with other neurite growth inducers (RA and Y-27632) used in present study.

## IV. 4.4. Statin modulates intracellular [$^3$H]dopamine uptake and the pharmacological properties of VMAT2 in SH-SY5Y cells

The statin-induced changes in neurite growth and in presynaptic marker expressions suggested a strong drive towards a dopaminergic phenotype with significant changes affecting several key elements of the DA transport cycle. These findings clearly supported further investigation of the potential impact of statins on the functionality of DA system. DAT, VMAT2 and SYNGR3 belong to a functional cycle for mobilizing different pools of DA in pre-synaptic structures DA (Eriksen et al., 2010).

Under our experimental conditions, the intracellular [$^3$H]DA uptake in SH-SY5Y cells was time-dependent and showed two kinetic phases reaching a first plateau at 15 min (S1) and a second at 60 min (S2) incubation time (velocity at S1 vs S2: 152±51 vs 59±54 pmol/mg of protein/min, $t_{(30)}$=4.872, *P*<0.0001) (Fig. 6a). [$^3$H]DA uptake kinetic was sensitive to the DAT inhibitor GBR-12935 and to the VMAT2 inhibitor reserpine (RSP). Full inhibition of DA uptake was observed by GBR-12935 at both S1 and S2 uptake phases while RSP was more efficacious at S2 (Fig. 6b). These results showed a differential kinetics of DA transport in SH-SY5Y cells which was fully sensitive to DAT function but with a major contribution of DA

vesicular transport at longer incubation times demonstrated by a VMAT2 inhibition sensitivity.

Treatment with lovastatin decreased the $V_{max}$ of [$^3$H]DA cellular uptake (S1) in SH-SY5Y cells at 24 h with a $V_{max}$=10.4±0.3 vs 7.2±0.5 pmol/mg protein/min in vehicle- and 50 µM lovastatin-treated cells respectively ($F_{(1,26)}$=28.91, *P*<0.0001) (Fig. 6c). This effect was also observed at 60min incubation time with a $V_{max}$=7.17±0.3 vs 4.4±0.2 pmol/mg protein/min for vehicle- and lovastatin-treatments respectively ($F_{(1,26)}$=53.08, *P*<0.0001) (Fig. 6d). Incubation of SH-SY5Y cells with lovastatin for longer time (48 h) induced similar reductions (Fig. 6e and f). The lovastatin-induce reduction of [$^3$H]DA uptake was dose-dependent and accounted for 31.4±5% and 34.1±10.5% of maximal effect at 24 h and 48 h post-treatment at S1 phase. Significant higher levels of reduction were observed at the S2 phase of [$^3$H]DA uptake with 37.5±3.8% (24 h) and 57.6±6.2% (48 h) (S1 vs S2 values; *t*-test $t_{(10)}$=2.3 *P*=0.042) (Fig. 6c-f). Pre-incubation of cells with selective transport inhibitors GBR-12935 did not reveal any difference in their inhibitory potency between lovastatin- or vehicle-treated cells at 24 h (Fig. 6g). GBR-12935 showed an $IC_{50}$=424±50 vs 348±77 nM in vehicle and lovastatin treated cells respectively ($F_{(1,32)}$=0.69, *P*=0.4106). Lack of change was also observed for VMAT2 inhibitor RSP ($IC_{50}$=916±140 vs 584±64 pM, $F_{(1,52)}$=2.11, *P*=0.1520) (Fig. 6h). In contrast, DA-transport inhibition by the VMAT2 allosteric inhibitor tetrabenazine (TBZ) displayed a biphasic curve ($n_H$=-0.39) in cells treated (24 h) with lovastatin (Fig. 6i). Nonlinear regression analysis revealed a high ($IC_{50}$=62.9±278 nM) and low ($IC_{50}$=26.6±19.4 µM) sensitivity inhibition component after statin treatment. TBZ-inhibition curve in vehicle treated cells showed one site ($IC_{50}$=20.4±5.6 µM) (Fig. 6i).

Longer lovastatin treatment times (48 h) induced the appearance of a RSP-insensitive [$^3$H]DA uptake components (Fig. 6k). RSP was less efficacious in for inhibiting [$^3$H]DA uptake in lovastatin-treated than in vehicle treated cells ($I_{max}$=77.9±2.1 vs 36.7±3.7%, $F_{(1,52)}$=12.34, *P*=0.0009) (Fig. 6k). Uptake inhibition curves also revealed a negative impact of lovastatin in TBZ efficacy ($I_{max}$=76.2±2.1 vs 47.5±3.5%, $F_{(1,64)}$=4.5, *P*=0.0377) (Fig. 6l). The effect of lovastatin treatment on RSP- and TBZ-inhibitory efficacy was clearly dose-dependent (Fig. 6k and l). Lovastatin (50 µM) also induced a decrease of the potency of VMAT2 inhibitors. Inhibition curves for [$^3$H]DA uptake significantly shifted to the right for RSP ($IC_{50}$=182±15.6 vs 699±192 pM, $F_{(1,56)}$=29.35, *P*<0.0001) and for TBZ ($IC_{50}$=4.6±0.9 vs 10.5±4.37 µM, $F_{(1,68)}$=4.4, *P*=0.0396) in lovastatin-treated compared to vehicle-treated cells

(Fig. 6k). The inhibitory efficacy of DAT inhibitor GBR-12935 remained unaffected by lovastatin treatment ($I_{max}$=99.7±1.2 vs 99.4±2.3%, $F_{(1,52)}$=0.02, *P*=0.8951) (Fig. 6j). In summary, lovastatin decreases total cellular levels of DA uptake and modulates VMAT2 pharmacological properties as evidenced by i) the transient appearance of a new TBZ-highly sensitive inhibition component, at shorter treatment time, and ii) an insensitivity to reference VMAT2 inhibitors at longer incubation times.

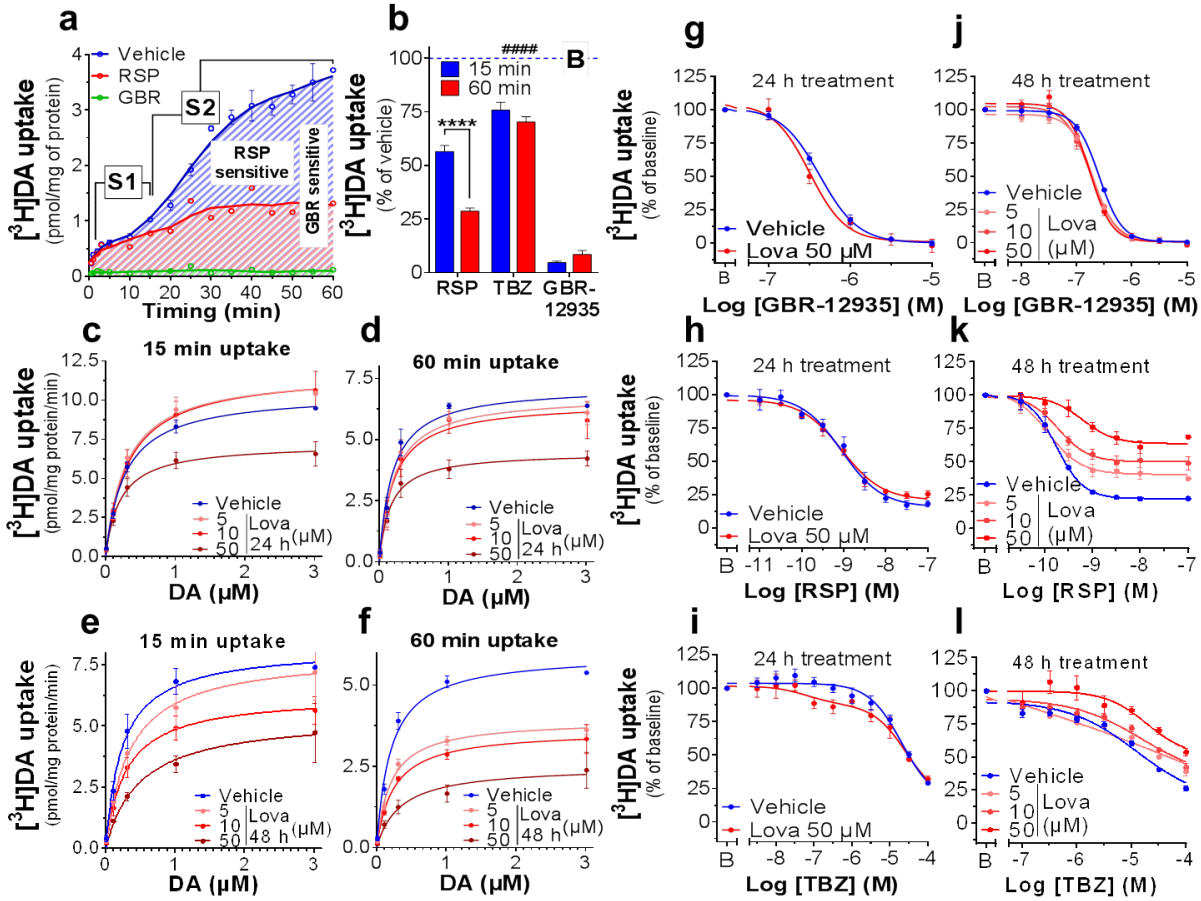


Figure 20. (Figure 6.) **Lovastatin decreases the dopamine (DA) uptake and changes the pharmacological properties of VMAT2 in SH-SY5Y cells**

(**a**) Representative kinetics of [$^3$H]DA uptake for 60 min in SH-SY5Y cells treated with reserpine (RSP) 1μM or GBR-12935 10 μM. (**b**) [$^3$H]DA uptake for 15 min or 60 min in cells pre-treated with RSP 1 μM, tetrabenazine (TBZ) 10 μM and GBR-12935 10 μM. Statistical analyses were performed by two-way ANOVA followed by Bonferroni post hoc test (treatment effect: $F_{(3,286)}$=1050, $P$<0.0001; Time: $F_{(1,286)}$=35.57, $P$<0.0001; Interaction: $F_{(3,286)}$=3.28, $P$<0.0001): all treatments reduced (####$P$<0.0001) the DA uptake compared to vehicle-treated cells; ****$P$<0.0001 for comparison between 15 min and 60min. (**c-f**) Saturation studies of [$^3$H]DA uptake for 15 min (**c** and **e**) or 60 min (**d** and **f**) in cells pre-treated with lovastatin (Lova) incubated 24 h (**c** and **d**) or 48 h (**e** and **f**). Michaelis–Menten equation was applied to saturation curves. g-l, dose-responses of GBR-12935, RSP and TBZ incubated 30 min followed by the [$^3$H]DA uptake for 60 min in cells pre-treated with lovastatin incubated 24 h (**g-i**) or 48 h (**j-l**). (**g, h, j, k** and **l**) Results were fitted by nonlinear regression with a 4PL equation. (**i**) Results of vehicle (two sites: $F_{(2,28)}$=1.64, $P$=0.21) and lovastatin (two site: $F_{(2,28)}$=3.27, $P$<0.05) treatments were fitted with one-site and two-site equation respectively. Results represent a means ± S.E.M. (n>3). B, basal level.

## IV. 4.5. VMAT2 overexpression in BE(2)-M17 cells induces changes of TBZ sensitivity similar to those observed with lovastatin

The statin-induced changes observed in DA transport of SH-SY5Y cells could result from an imbalance of the different elements of the DA transport cycle due to changes in protein expressions. To investigate this hypothesis, we assessed the impact of the ectopic overexpression of VMAT2 on the DA transport in a heterologous expression system. Preliminary transfection experiments using SH-SY5Y cells did not lead to a suitable VMAT2 overexpression level. Subsequently, we selected the BE(2)-M17 neuroblastoma cells, which also display a pronounced dopaminergic phenotype. Immunoblot analysis confirmed the expression of VMAT2 in wild-type BE(2)-M17 cells (Fig. 7a). VMAT-2 gene transfection induced a significant increase in the levels of native and glycosylated VMAT2 protein forms (Fig. 7a). DA-transport experiments revealed an increase in DA-uptake (248±16.2 %) in VMAT2-transfected cells versus the corresponding wild-type cells (Fig. 7b). Pre-incubation of untransfected cells with selective transport inhibitors showed a potent and dose-dependent inhibition of [$^3$H]DA transport by RSP and TBZ (Fig. 7c and d). In VMAT2-transfected BE(2)-M17 cells, RSP showed a modest shift of its inhibitory potency compared with untransfected cells ($IC_{50}$=1.53±0.23 vs 5.25±0.57 nM, $F_{(1,76)}$=49.41, $P$<0.0001) (Fig. 7c) while TBZ displayed a clear biphasic inhibition of [$^3$H]DA transport compared with untransfected cells ($n_H$=-0.62±0.12 vs -0.19±0.12, $F_{(1,120)}$=6.05, $P$=0.0154) (Fig. 7d). Nonlinear regression demonstrated no changes of the TBZ-sensitive low-affinity component between untransfected and transfected cells ($IC_{50}$=25.8±11.8 vs 18.8±12.3 µM, $F_{(1,110)}$=0.29, $P$=0.5851) while an increase in the efficacy for the high affinity site was observed (Fig. 7d) ($IC_{50}$=37.8±75 vs 27.4±12.9 nM, $F_{(1,110)}$=0.073, $P$=0.7864; $I_{high\text{-}affinity\text{-}site}$=101.3±5.6 vs 76.4±6.2%, $t$-test$_{(8)}$=2.93, $P$=0.0187) (Fig. 7d). Overall, these results show that VMAT2 overexpression in BE(2)-M17 cells increased the DA uptake level and modulate VMAT2 sensitivity to TBZ mimicking the impact of statins found on VMAT2 in SH-SY5Y cells.

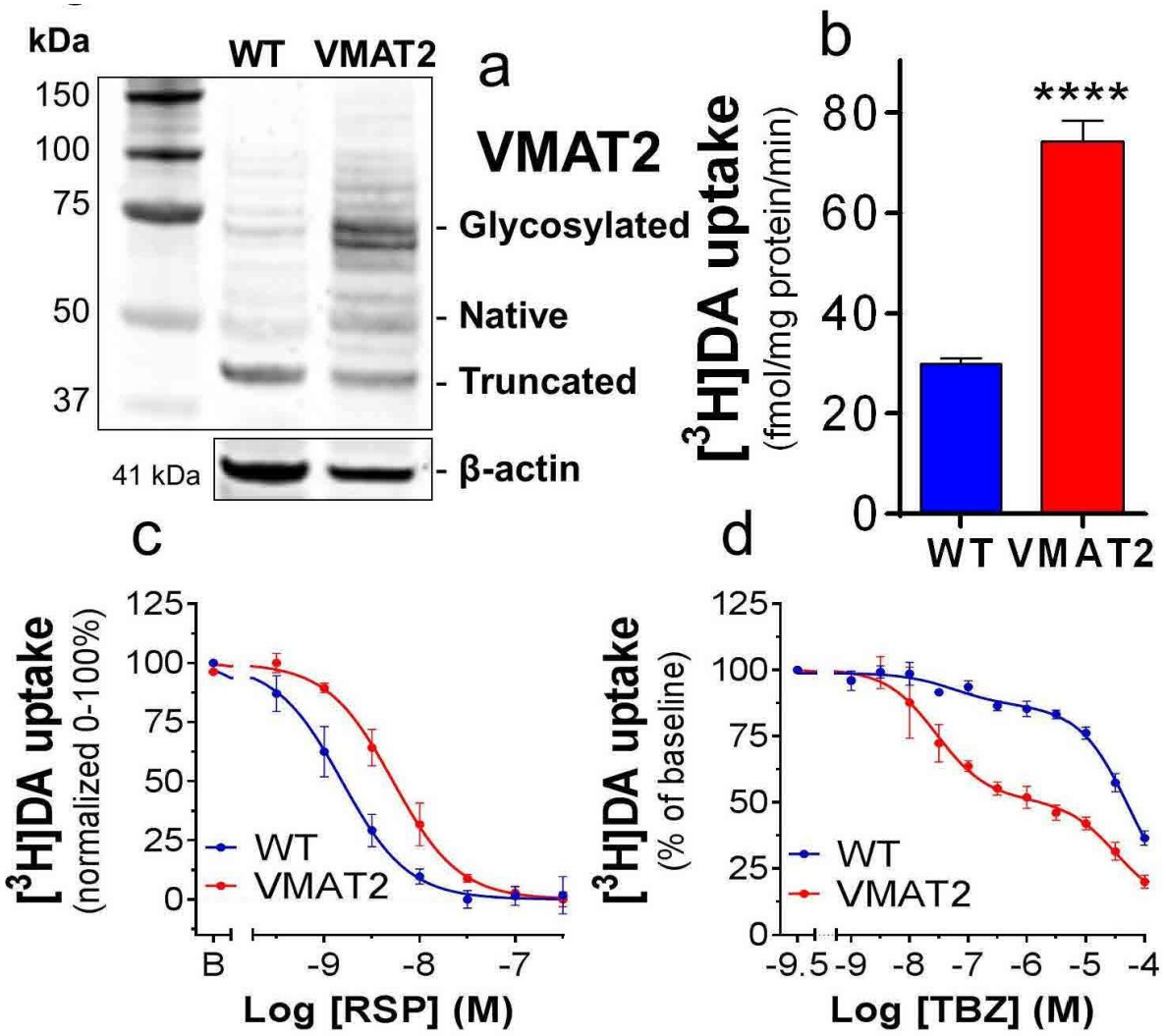


Figure 21. (Figure 7.) **VMAT2 overexpression increases the capacity of dopamine transport and modulates the sensitivity of VMAT2 inhibitors in BE(2)-M17 cells**

(**a**) Representative immunoblot of VMAT2 revealing the truncated (45 kDa), the native (55 kDa) and the glycosylated (75 kDa) forms of VMAT2 in wild-type (WT) and VMAT2 transfected cells. b, [$^3$H]DA uptake in WT and VMAT2 transfected cells. Statistical analysis was performed by student's t-test ($t_{(26)}$=10.54, $P$<0.0001, n=14). (**c-d**) Dose-responses of reserpine (**c**, RSP) and tetrabenazine (**d**, TBZ) pre-incubated 30 min followed by the [$^3$H]DA uptake for 60min in WT and VMAT2 transfected cells. c, RSP results were fitted by nonlinear regression with a normalized equation. (**d**) Two-site fitting was applied for WT ($F_{(2,53)}$=3.96, $P$<0.05) and VMAT2 transfected cells treated with TBZ ($F_{(2,51)}$=17.88, $P$<0.0001) after comparison with one-site fitting. Results represent a means ± S.E.M. (n=3-5). B, basal level.

## IV. 4.6. Nuclear translocation of SREBP-1 is triggered by statins in SH-SY5Y cells

The activation of SREBP transcription is a cell signalling pathway constituting a direct feedback loop for the transcriptional modulation of key enzymes involved in the mevalonate pathway (Fig. 1i) (Kallin et al., 2007). We aimed to determine the potential activation of SREBP-1 transcription factor by statins. Immunoblot analysis of vehicle-treated SH-SY5Y cells revealed the expression of full and mature (translocated) forms of SREBP-1 (Fig. 8a). The incubation of cultured cells with lovastatin (24 h) induced an increase in the level of the mature form of SREBP-1 (Fig. 8a). Immunofluorescence detection and high-content imaging analysis confirmed that lovastatin induced a dose- and time-dependent increase in the number of cells with positive nuclear staining of SREBP-1 antibody (Fig. 8b) as well as an increase in the fluorescence intensity ratio (cytoplasm/nucleus) (Fig. 8c and d). The results showed lovastatin-induced translocation of the SREBP-1 protein from the cytosol to the nucleus as early as 6 h after treatment. The maximal effect of the statin was reached 48 h after treatment with an $EC_{50}$ of 4.1±1.67 µM (Fig. 8c and d). Similar effects were observed with the simvastatin (Fig. 8d). In conclusion, statins lead an increase in SREBP translocation into the nucleus in SH-SY5Y cells concomitantly to an increase in neurite growth, gene expressions of dopaminergic markers and modulation of DA transport.

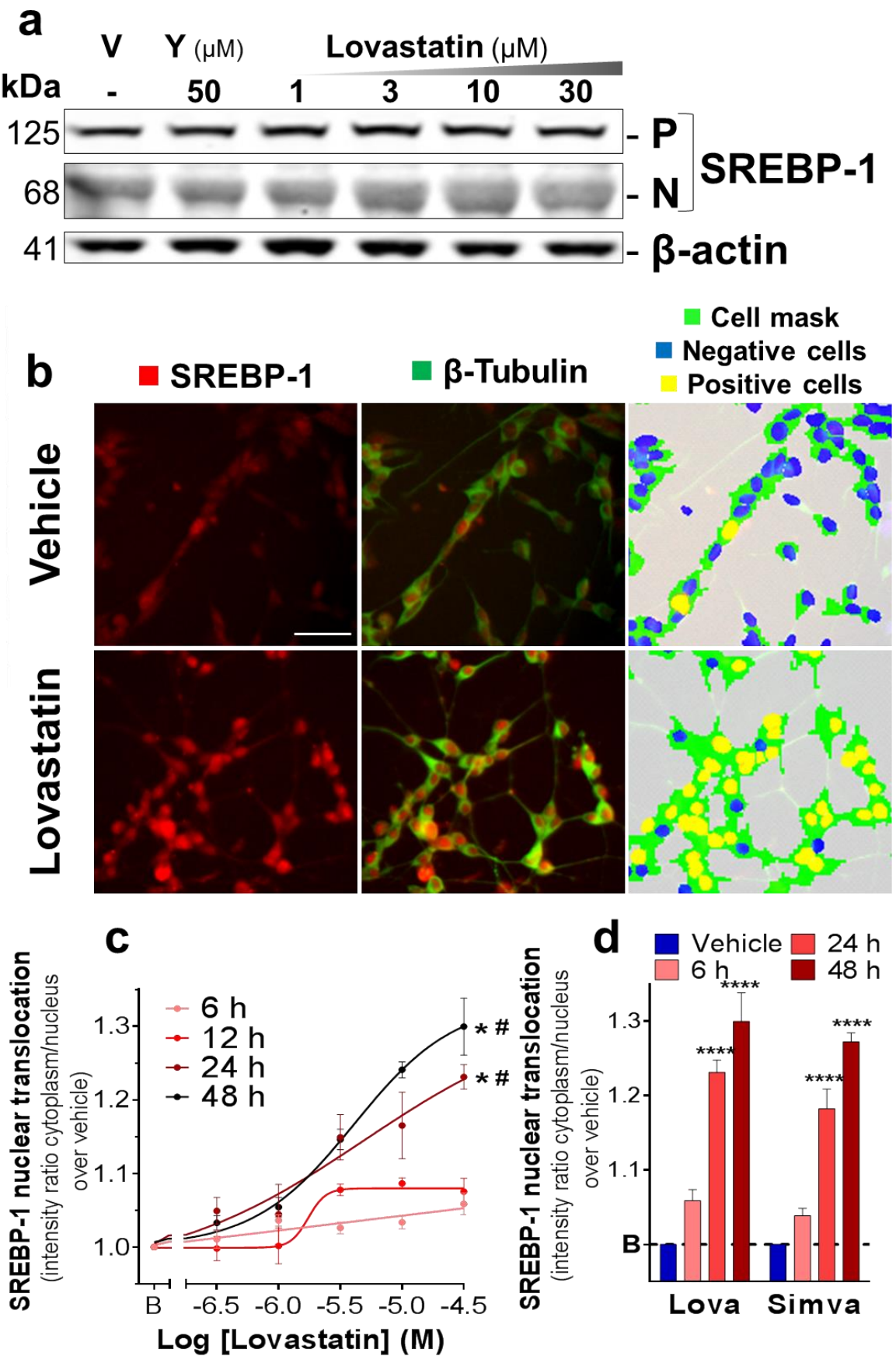


Figure 22. (Figure 8.) **Statins lead the nuclear translocation of SREBP-1 in SH-SY5Y cells**

(**a**) Representative immunoblot of SREBP-1 nuclear translocation revealing the precursor of SREBP-1 (P SREBP-1, 125 kDa) and mature nuclear SREBP-1 (N SREBP-1, 68 kDA) in cells treated with lovastatin dose-response or Y-27632 (Y) 50 µM. (**b)** Representative immunofluorescence images of SREBP-1 and βIII-tubulin staining in SH-SY5Y cells. Image Processing and classification of positive cells for the nuclear translocation of SREBP-1. (**c**) Effect of lovastatin dose-response kinetics (6 to 48 h) on SREBP-1 nuclear translocation. Results were fitted by nonlinear regression with a 4PL equation (lovastatin: treatment effect: $F_{(5,46)}$=19.78, $P$<0.0001; Time: $F_{(3,46)}$=17.68, $P$<0.0001; Interaction: $F_{(15,46)}$=2.95, $P$<0.0024). (**d**) Kinetics effect of lovastatin and simvastatin (Simva) 30µM treatments on SREBP-1 nuclear translocation (lovastatin vs simvastatin effect: $F_{(1,4)}$=1.64, $P$=0.2696; Time: $F_{(3,12)}$=143.7, $P$<0.0001; Interaction: $F_{(3,12)}$=0.82, $P$<0.5083). Results represent the mean ± S.E.M. (n=3-7). Statistical analyses were performed by two-way ANOVA followed by Bonferroni post hoc test: */# were represented when a point of the curve was at least $P$<0.05 compared to baseline (*) or 6 h of treatment (#). In kinetics bar graph ****$P$<0.0001, vs corresponding vehicle treated cells. B, basal level. Scale bar indicates 25 µm.

# IV. 5. Discussion

We here report that treatment of SH-SY5Y cells with statins induces (i) a neurotrophic effect on synaptic structures reflected by an increase in neurite length and complexity of neurite branching (ii) a reversal of neurite retraction mediated by PI3K inhibition (iii) an induction of the dopaminergic phenotype by increasing transcription and protein levels of the pre-synaptic markers (SV2C, SV2A, VMAT2 and SYNGR3) and by increasing protein levels of TH and DAT (iv) a modulatory effect on the DA transport system characterized by a decrease the DA uptake and by changes of the pharmacological properties of VMAT2 and (v) an activation of SREBP pathway which could participates to the observed trophic effects.

Neurotrophic action of statins observed in our study affected neurite branching rather than elongation of primary neurites of SH-SY5Y. Our data agree and extend previous findings showing that simvastatin induces an overall neurite growth in SH-SY5Y cells (Raina et al., 2013). The increased branching induced by statin strength data in primary cortical neurons showing the induction of neuritic terminal branching by atorvastatin (Watanabe et al., 2012). Statin-induced neurite growth clearly differentiates from the neurotrophic effect observed by ROCK-inhibition (Racchetti et al., 2010) or RAR stimulation (Constantinescu et al., 2007; Lopes et al., 2010). These neurotrophic compounds only affected overall elongations while statins increased network complexity. Moreover, our data suggest that statin's effect is likely independent from RhoA or PI3K signalling pathways (Evangelopoulos et al., 2009; Jin et al., 2012; Raina et al., 2013; Samuel et al., 2014). Interestingly, our findings show that statins are able to reverse the neurite retraction induced by PI3K-inhibition suggesting a neuroregenerative potential. Pathophysiology of PD is characterized by cellular mechanisms leading to dopaminergic cell death and axonal terminal degeneration (Coleman, 2005).

Compelling evidences suggest that neurodegeneration begins at the presynaptic terminal in the distal axons followed by a retrograde degeneration up to the soma (Coleman, 2005). Actually, at early stages of PD, only 30% of DA neurons are lost in substantia nigra while 50-60% of axon terminals are lost in the striatum (Cheng et al., 2010). Moreover, axonal degeneration and neurite retraction have been demonstrated in toxin-induced, α-synuclein overexpression and mutated leucine-rich repeat kinase 2 (Chan et al., 2011; Winner et al., 2011; Koch et al., 2015). We could hypothesize that statins might counteract PD neurodegeneration through (i) a blockade of retrograde axonal degeneration and/or (ii) by promoting neurite branching. Other neurotrophic principles and mechanisms such as GDNF factor (Rosenblad et al., 1999), ROCK inhibition (Zhao et al., 2015) or ROCK downregulation (Saal et al., 2015) have been shown to promoting axonal regeneration in models for PD.

In addition to the neurotrophic effects, we observed a statin-induced up-regulation of synaptic vesicular elements, which may also support potential neuroprotective effect in PD. Several reports have previously suggested the interest of VMAT2 as a target for neuroprotection in PD (Guillot and Miller, 2009; Yulug et al., 2015). It has been proposed that the decline of VMAT2 expression could be a pathological event preceding the nigrostriatal dopaminergic degeneration (Chen et al., 2008). Moreover, the decrease of VMAT2 activity enhanced the susceptibility to MPTP neurotoxicity and induced a PD-like pathology in VMAT2 knockout animals (Takahashi et al., 1997; Gainetdinov et al., 1998) and in animals treated with VMAT inhibitor RSP (Staal and Sonsalla, 2000). In this context, VMAT2 up-regulation induced by statins may facilitate the transport of excessive intracytosolic DA or other DA-related neurotoxic agents into vesicles which, in turn, may reduce oxidative stress and subsequent cell toxicity (Speciale et al., 1998; Chen et al., 2005; Brighina et al., 2013; Lohr et al., 2014). Similarly, the statin-induced expression of the putative transporter SV2C might also contribute to modulate cytosolic DA levels (Dardou et al., 2011, 2013).

The present data demonstrate that statin-treatment triggered nuclear translocation of SREBP-1 in SH-SY5Y cells, a result in agreement with previous reports on statin-induced SREBP-transcriptional activity in non-neuronal cells (Roglans et al., 2002; Rise et al., 2007). The nuclear translocation of SREBP-1 is concomitant to neurotrophic and phenotypic effects on the dopaminergic markers, pointing out this transcription factors as downstream regulator of those effects. Currently, it has been shown that the liver X receptor, a transcriptional

regulator of SREBP-1 genes (Chen et al., 2004; Cha and Repa, 2007) controls the midbrain neuronal dopaminergic phenotype and promotes differentiation of embryonic stem cells towards a TH-positive and VMAT2-positive phenotype (Sacchetti et al., 2009; Theofilopoulos et al., 2013). Interestingly, a previous study in non-neuronal cells (Hep G2) reported an increase in SV2A mRNA levels induced by lovastatin and by SREBP-1 transfection. Statin-induced expression of vesicular transporters reported here strongly suggest a potential common downstream pathway for both neuronal and non-neuronal cells (Kallin et al., 2007). Whether the statin-induced regulation of the presynaptic genes is a consequence of direct activation of SREBP-dependent transcriptional activity or other downstream pathway remains to be demonstrated.

The impact of statins in [$^3$H]DA transport in SH-SY5Y cells strongly suggest a dynamic sensitivity of the DA-transport system, subsequent to changes in the expression levels of presynaptic proteins. The statin-induced effects on DA transport activity may potentially impact the neurodegenerative processes in PD by interfering with several mechanisms. A reduction of DA transport associated to an increase in VMAT2 and SYNGR3 expressions may decrease cytosolic DA content, and reduce uptake of potential extracellular neurotoxic agents thus reducing intracellular oxidative stress (Kurosaki et al., 2003; Rappold et al., 2011; Segura-Aguilar et al., 2014; Masoud et al., 2015). Moreover, a reduced DA uptake may prolong the exposure to DA in the synaptic cleft, thus sustaining post-synaptic stimulation that would help to manage severity of the motor symptoms (Bezard and Gross, 1998; Bezard et al., 2003). The statin-induced expression of key elements of the DA transport cycle such as SYNGR3 could explain the observed changes in DA transport. Actually, previous studies showed that overexpression of SYNGR3 triggered changes in the DA transport system by regulating VMAT2 and DAT function (Egana et al., 2009). Given the potential reduction of cholesterol levels in our conditions, a direct effect of low cholesterol in the DA transport system could not be ignored. It has been shown that low levels of cholesterol impact DAT time residency in membrane lipid rafts, DAT internalization process and subsequently cause a decrease in DA uptake (Foster et al., 2008; Jones et al., 2012).

Our findings suggest the interest of statins for the treatment of neurodegeneration in PD. However, the therapeutic potential of statins in PD patients remains to be demonstrated.

In conclusion, the present data strengthen the therapeutic potential of statins in PD by unravelling (i) their trophic effects including protection and regeneration of neurites, (ii) their ability to induce the dopaminergic phenotype and (iii) their modulation of DA transport. Further investigation of the cellular and molecular effects of statins in the synaptic machinery may extend the understanding of their downstream signalling mechanisms and of the proposed therapeutic uses for these molecules.

# IV. 6. References

Bar-On P, Crews L, Koob AO, Mizuno H, Adame A, Spencer B, Masliah E (2008) Statins reduce neuronal alpha-synuclein aggregation in in vitro models of Parkinson's disease. J Neurochem 105:1656–1667.

Bezard E, Gross CE (1998) Compensatory mechanisms in experimental and human parkinsonism: towards a dynamic approach. Prog Neurobiol 55:93–116.

Bezard E, Gross CE, Brotchie JM (2003) Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. Trends Neurosci 26:215–221.

Brighina L, Riva C, Bertola F, Saracchi E, Fermi S, Goldwurm S, Ferrarese C (2013) Analysis of vesicular monoamine transporter 2 polymorphisms in Parkinson's disease. Neurobiol Aging 34:1712 e9–e13.

Castro AA, Wiemes BP, Matheus FC, Lapa FR, Viola GG, Santos AR, Tasca CI, Prediger RD (2013) Atorvastatin improves cognitive, emotional and motor impairments induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats, an experimental model of Parkinson's disease. Brain Res 1513:103–116.

Cha JY, Repa JJ (2007) The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. J Biol Chem 282:743–751.

Chan D, Citro A, Cordy JM, Shen GC, Wolozin B (2011) Rac1 Protein Rescues Neurite Retraction Caused by G2019S Leucine-rich Repeat Kinase 2 (LRRK2). J Biol Chem 286:16140–16149.

Chen CX, Huang SY, Zhang L, Liu YJ (2005) Synaptophysin enhances the neuroprotection of VMAT2 in MPP+-induced toxicity in MN9D cells. Neurobiol Dis 19:419–426.

Cheng H-C, Ulane CM, Burke RE (2010) Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol 67:715–725.

Chen G, Liang G, Ou J, Goldstein JL, Brown MS (2004) Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc Natl Acad Sci U A 101:11245–11250.

Chen M-K, Kuwabara H, Zhou Y, Adams RJ, Brašić JR, McGlothan JL, Verina T, Burton NC, Alexander M, Kumar A, Wong DF, Guilarte TR (2008) VMAT2 and dopamine neuron loss in a primate model of Parkinson's disease. J Neurochem 105:78–90.

Coleman M (2005) Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6:889–898.

Constantinescu R, Constantinescu AT, Reichmann H, Janetzky B (2007) Neuronal differentiation and long-term culture of the human neuroblastoma line SH-SY5Y. J Neural Transm Suppl:17–28.

Dardou D, Dassesse D, Cuvelier L, Deprez T, De Ryck M, Schiffmann SN (2011) Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res 1367:130–145.

Dardou D, Monlezun S, Foerch P, Courade JP, Cuvelier L, De Ryck M, Schiffmann SN (2013) A role for Sv2c in basal ganglia functions. Brain Res 1507:61–73.

Egana LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Pena K, Quiroz M, Hong WC, Dorostkar MM, Janz R, Sitte HH, Torres GE (2009) Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. J Neurosci 29:4592–4604.

Evangelopoulos ME, Weis J, Kruttgen A (2009) Mevastatin-induced neurite outgrowth of neuroblastoma cells via activation of EGFR. J Neurosci Res 87:2138–2144.

Foster JD, Adkins SD, Lever JR, Vaughan RA (2008) Phorbol ester induced trafficking-independent regulation and enhanced phosphorylation of the dopamine transporter associated with membrane rafts and cholesterol. J Neurochem 105:1683–1699.

Gainetdinov RR, Fumagalli F, Wang Y-M, Jones SR, Levey AI, Miller GW, Caron MG (1998) Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. J Neurochem 70:1973–1978.

Gao X, Simon KC, Schwarzschild MA, Ascherio A (2012) Prospective study of statin use and risk of Parkinson disease. Arch Neurol 69:380–384.

Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, Pahan K (2009) Simvastatin inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson's disease. J Neurosci 29:13543–13556.

Guillot TS, Miller GW (2009) Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. Mol Neurobiol 39:149–170.

Janowsky A, Neve K, Eshleman AJ (2001) Uptake and release of neurotransmitters. Curr Protoc Neurosci Chapter 7:Unit7 9.

Jin Y, Sui HJ, Dong Y, Ding Q, Qu WH, Yu SX, Jin YX (2012) Atorvastatin enhances neurite outgrowth in cortical neurons in vitro via up-regulating the Akt/mTOR and Akt/GSK-3beta signaling pathways. Acta Pharmacol Sin 33:861–872.

Jones KT, Zhen J, Reith MEA (2012) Importance of cholesterol in dopamine transporter function. J Neurochem 123:700–715.

Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferre P, Heldin CH, Delzenne NM, Demoulin JB (2007) SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 gamma. J Lipid Res 48:1628–1636.

Kim JB, Spiegelman BM (1996) ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev 10:1096–1107.

Koch JC, Bitow F, Haack J, d'Hedouville Z, Zhang J-N, Tönges L, Michel U, Oliveira LMA, Jovin TM, Liman J, Tatenhorst L, Bähr M, Lingor P (2015) Alpha-Synuclein affects neurite morphology, autophagy, vesicle transport and axonal degeneration in CNS neurons. Cell Death Dis 6:e1811.

Koob AO, Ubhi K, Paulsson JF, Kelly J, Rockenstein E, Mante M, Adame A, Masliah E (2010) Lovastatin ameliorates alpha-synuclein accumulation and oxidation in transgenic mouse models of alpha-synucleinopathies. Exp Neurol 221:267–274.

Kumar A, Sharma N, Gupta A, Kalonia H, Mishra J (2012) Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. Brain Res 1471:13–22.

Kurosaki R, Muramatsu Y, Watanabe H, Michimata M, Matsubara M, Imai Y, Araki T (2003) Role of dopamine transporter against MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) neurotoxicity in mice. Metab Brain Dis 18:139–146.

Lee YC, Lin CH, Wu RM, Lin MS, Lin JW, Chang CH, Lai MS (2013) Discontinuation of statin therapy associates with Parkinson disease: a population-based study. Neurology 81:410–416.

Li L, Zhang W, Cheng S, Cao D, Parent M (2012) Isoprenoids and related pharmacological interventions: potential application in Alzheimer's disease. Mol Neurobiol 46:64–77.

Lohr KM, Bernstein AI, Stout KA, Dunn AR, Lazo CR, Alter SP, Wang M, Li Y, Fan X, Hess EJ, Yi H, Vecchio LM, Goldstein DS, Guillot TS, Salahpour A, Miller GW (2014) Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. Proc Natl Acad Sci U A 111:9977–9982.

Lopes FM, Schroder R, da Frota ML, Zanotto-Filho A, Muller CB, Pires AS, Meurer RT, Colpo GD, Gelain DP, Kapczinski F, Moreira JC, Fernandes Mda C, Klamt F (2010) Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res 1337:85–94.

Lotharius J, Brundin P (2002) Pathogenesis of parkinson's disease: dopamine, vesicles and α-synuclein. Nat Rev Neurosci 3:932–942.

Lu D, Qu C, Goussev A, Jiang H, Lu C, Schallert T, Mahmood A, Chen J, Li Y, Chopp M (2007) Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J Neurotrauma 24:1132–1146.

Malfitano AM, Marasco G, Proto MC, Laezza C, Gazzerro P, Bifulco M (2014) Statins in neurological disorders: an overview and update. Pharmacol Res 88:74–83.

Masoud ST, Vecchio LM, Bergeron Y, Hossain MM, Nguyen LT, Bermejo MK, Kile B, Sotnikova TD, Siesser WB, Gainetdinov RR, Wightman RM, Caron MG, Richardson JR, Miller GW, Ramsey AJ, Cyr M,

Salahpour A (2015) Increased expression of the dopamine transporter leads to loss of dopamine neurons, oxidative stress and l-DOPA reversible motor deficits. Neurobiol Dis 74:66–75.

Pooler AM, Xi SC, Wurtman RJ (2006) The 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitor pravastatin enhances neurite outgrowth in hippocampal neurons. J Neurochem 97:716–723.

Racchetti G, Lorusso A, Schulte C, Gavello D, Carabelli V, D'Alessandro R, Meldolesi J (2010) Rapid neurite outgrowth in neurosecretory cells and neurons is sustained by the exocytosis of a cytoplasmic organelle, the enlargeosome. J Cell Sci 123:165–170.

Raina V, Gupta S, Yadav S, Surolia A (2013) Simvastatin induced neurite outgrowth unveils role of cell surface cholesterol and acetyl CoA carboxylase in SH-SY5Y cells. PLoS One 8:e74547.

Rappold PM, Cui M, Chesser AS, Tibbett J, Grima JC, Duan L, Sen N, Javitch JA, Tieu K (2011) Paraquat neurotoxicity is mediated by the dopamine transporter and organic cation transporter-3. Proc Natl Acad Sci 108:20766–20771.

Reiss AB, Wirkowski E (2007) Role of HMG-CoA reductase inhibitors in neurological disorders : progress to date. Drugs 67:2111–2120.

Rise P, Ghezzi S, Carissimi R, Mastromauro F, Petroni A, Galli C (2007) Delta5 desaturase mRNA levels are increased by simvastatin via SREBP-1 at early stages, not via PPARalpha, in THP-1 cells. Eur J Pharmacol 571:97–105.

Robin NC, Agoston Z, Biechele TL, James RG, Berndt JD, Moon RT (2014) Simvastatin Promotes Adult Hippocampal Neurogenesis by Enhancing Wnt/β-Catenin Signaling. Stem Cell Rep 2:9–17.

Roglans N, Verd JC, Peris C, Alegret M, Vazquez M, Adzet T, Diaz C, Hernandez G, Laguna JC, Sanchez RM (2002) High doses of atorvastatin and simvastatin induce key enzymes involved in VLDL production. Lipids 37:445–454.

Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Björklund A (1999) Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. Eur J Neurosci 11:1554–1566.

Roy A, Pahan K (2011) Prospects of statins in Parkinson disease. Neuroscientist 17:244–255.

Saal K-A, Koch JC, Tatenhorst L, Szegő ÉM, Ribas VT, Michel U, Bähr M, Tönges L, Lingor P (2015) AAV.shRNA-mediated downregulation of ROCK2 attenuates degeneration of dopaminergic neurons in toxin-induced models of Parkinson's disease in vitro and in vivo. Neurobiol Dis 73:150–162.

Sacchetti P, Sousa KM, Hall AC, Liste I, Steffensen KR, Theofilopoulos S, Parish CL, Hazenberg C, Richter LA, Hovatta O, Gustafsson JA, Arenas E (2009) Liver X receptors and oxysterols promote ventral midbrain neurogenesis in vivo and in human embryonic stem cells. Cell Stem Cell 5:409–419.

Samuel F, Reddy J, Kaimal R, Segovia V, Mo H, Hynds DL (2014) Inhibiting geranylgeranylation increases neurite branching and differentially activates cofilin in cell bodies and growth cones. Mol Neurobiol 50:49–59.

Sato-Suzuki I, Murota S (1996) Simvastatin inhibits the division and induces neurite-like outgrowth in PC12 cells. Neurosci Lett 220:21–24.

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108.

Schulz JG, Bosel J, Stoeckel M, Megow D, Dirnagl U, Endres M (2004) HMG-CoA reductase inhibition causes neurite loss by interfering with geranylgeranylpyrophosphate synthesis. J Neurochem 89:24–32.

Segura-Aguilar J, Paris I, Munoz P, Ferrari E, Zecca L, Zucca FA (2014) Protective and toxic roles of dopamine in Parkinson's disease. J Neurochem 129:898–915.

Shao W, Espenshade PJ (2012) Expanding roles for SREBP in metabolism. Cell Metab 16:414–419.

Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N (1999) Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 274:35832–35839.

Speciale SG, Liang CL, Sonsalla PK, Edwards RH, German DC (1998) The neurotoxin 1-methyl-4-phenylpyridinium is sequestered within neurons that contain the vesicular monoamine transporter. Neuroscience 84:1177–1185.

Staal RG, Sonsalla PK (2000) Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. J Pharmacol Exp Ther 293:336–342.

Suzuki R, Lee K, Jing E, Biddinger SB, McDonald JG, Montine TJ, Craft S, Kahn CR (2010) Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism. Cell Metab 12:567–579.

Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR (1997) VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci 94:9938–9943.

Theofilopoulos S, Wang Y, Kitambi SS, Sacchetti P, Sousa KM, Bodin K, Kirk J, Salto C, Gustafsson M, Toledo EM, Karu K, Gustafsson JA, Steffensen KR, Ernfors P, Sjovall J, Griffiths WJ, Arenas E (2013) Brain endogenous liver X receptor ligands selectively promote midbrain neurogenesis. Nat Chem Biol 9:126–133.

van der Most PJ, Dolga AM, Nijholt IM, Luiten PG, Eisel UL (2009) Statins: mechanisms of neuroprotection. Prog Neurobiol 88:64–75.

Wahner AD, Bronstein JM, Bordelon YM, Ritz B (2008) Statin use and the risk of Parkinson disease. Neurology 70:1418–1422.

Wang Q, Yan J, Chen X, Li J, Yang Y, Weng J, Deng C, Yenari MA (2011) Statins: multiple neuroprotective mechanisms in neurodegenerative diseases. Exp Neurol 230:27–34.

Watanabe T, Yasutaka Y, Nishioku T, Nakashima A, Futagami K, Yamauchi A, Kataoka Y (2012) Atorvastatin stimulates neuroblastoma cells to induce neurite outgrowth by increasing cellular prion protein expression. Neurosci Lett 531:114–119.

Winner B, Melrose HL, Zhao C, Hinkle KM, Yue M, Kent C, Braithwaite AT, Ogholikhan S, Aigner R, Winkler J (2011) Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. Neurobiol Dis 41:706–716.

Wolozin B, Wang SW, Li NC, Lee A, Lee TA, Kazis LE (2007) Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. BMC Med 5:20.

Xu YQ, Long L, Yan JQ, Wei L, Pan MQ, Gao HM, Zhou P, Liu M, Zhu CS, Tang BS, Wang Q (2013) Simvastatin induces neuroprotection in 6-OHDA-lesioned PC12 via the PI3K/AKT/caspase 3 pathway and anti-inflammatory responses. CNS Neurosci Ther 19:170–177.

Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, Xiong Z, Deng C, Huang XF, Yenari MA, Yang YG, Ying W, Wang Q (2011) Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. PLoS One 6:e20945.

Yulug B, Hanoglu L, Kilic E (2015) The neuroprotective role of vesicular monoamine transporter 2 in neurodegenerative diseases. Med Chem 11:104–108.

Zhao Y, Zhang Q, Xi J, Li Y, Ma C, Xiao B (2015) Multitarget intervention of Fasudil in the neuroprotection of dopaminergic neurons in MPTP-mouse model of Parkinson's disease. J Neurol Sci 353:28–37.

# IV. 7. Supplemental data

Table 8. (Supplemental Table 1) **Pharmacological parameters of the Y-27632, lovastatin and wortmannin dose-responses alone or in combination on the total neurite length in SH-SY5Y cells**

| Total length of neurite outgrowth per cell: pharmacologic parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1st drug** (µM) | | **Basal value** without dose-response (µm) | **2nd drug** Dose-response | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (µm) | **$E_{max}$** (Δ µm over basal value) |
| **Lova** | - | 28.6 ± 1.5 | **Y-27632** | 3.2 ± 0.3 | 0.9 ± 0.1 | 70.5 ± 3.2 | 41.9 ± 3.5 |
| | 13 | 31.0 ± 0.4 | | 1.8 ± 0.2 * | 1.0 ± 0.1 | 70.2 ± 3.4 | 39.2 ± 3.4 |
| | 25 | 37.0 ± 1.3 * | | 2.1 ± 0.2 | 0.4 ± 0.2 | 76.6 ± 3.3 | 39.6 ± 3.5 |
| | 50 | 45.2 ± 2.3 *** | | 3.8 ± 0.5 | 0.4 ± 0.2 | 82.8 ± 2.2 | 37.6 ± 3.2 |
| | | $F_{(3,8)}$=23.3 *P*=0.0003 | | $F_{(3,8)}$=8.3 *P*=0.0076 | $F_{(3,8)}$=3.7 *P*=0.0591 | $F_{(3,8)}$=3.8 *P*=0.0585 | $F_{(3,8)}$=0.3 *P*=0.8440 |
| **Wort** | - | 28.5 ± 2.4 | **Lova** | 17.3 ± 2.4 | 2.3 ± 0.6 | 44.1 ± 1.2 | 15.5 ± 2.7 |
| | 2.5 | 20.0 ± 1.3 * | | 16.6 ± 1.9 | 2.3 ± 0.5 | 36.6 ± 1.7 * | 16.8 ± 2.1 |
| | 5 | 18.0 ± 1.1 ** | | 17.0 ± 1.7 | 2.6 ± 0.5 | 35.2 ± 1.9 * | 17.2 ± 2.2 |
| | 10 | 15.3 ± 1.7 ** | | 17.5 ± 2.4 | 1.9 ± 0.4 | 33.8 ± 1.5 ** | 18.5 ± 2.3 |
| | | $F_{(3,8)}$=11.26 *P*=0.0003 | | $F_{(3,8)}$=0.03 *P*=0.9909 | $F_{(3,8)}$=0.25 *P*=0.8544 | $F_{(3,8)}$=8.2 *P*=0.0078 | $F_{(3,8)}$=23.1 *P*=0.0003 |
| **Wort** | - | 27.6 ± 1.8 | **Y-27632** | 4.5 ± 0.4 | 1.0 ± 0.1 | 72.3 ± 4.9 | 44.6 ± 5.3 |
| | 2.5 | 19.3 ± 1.9 * | | 3.1 ± 0.3 | 1.0 ± 0.1 | 61.0 ± 3.7 | 41.7 ± 4.2 |
| | 5 | 16.1 ± 1.3 ** | | 2.9 ± 0.2 * | 1.0 ± 0.1 | 56.9 ± 1.6 | 40.8 ± 2.1 |
| | 10 | 20.2 ± 1.9 | | 4.4 ± 0.4 | 1.1 ± 0.1 | 61.5 ± 3.2 | 41.3 ± 3.7 |
| | | $F_{(3,8)}$=7.9 *P*=0.0093 | | $F_{(3,8)}$=6.3 *P*=0.0168 | $F_{(3,8)}$=0.2 *P*=0.8980 | $F_{(3,8)}$=3.4 *P*=0.0725 | $F_{(3,8)}$=0.2 *P*=0.9053 |

Compounds were incubated 24 h in SH-SY5Y cells.
Results represent the means ± S.E.M. (n=3) and pharmacological parameters were determined by a nonlinear regression with a 4PL or normalized fitting. Statistical analysis were performed by one-way ANOVA followed by Bonferroni post hoc test: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 were represented for comparison between vehicle (without 1st drug) and 1st drug treatment (± dose-response of 2nd drug).
See figure 1n, o and q for curves.
Lova, Lovastatin; Wort, wortmannin; Δ, difference.

Table 9. (Supplemental Table 2) **Pharmacological parameters of the Y-27632, lovastatin and wortmannin dose-responses alone or in combination on the primary and secondary neurite length in SH-SY5Y cells**

| Length of the primary and secondary neurites : pharmacologic parameters | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st drug (µM) | | Basal value without dose-response (µm) | | 2nd drug dose-response | EC50 (µM) | | nh | | Emax (µm) | | Emax (µm Δ over basal value) | |
| **Primary neurite length** | | | | | | | | | | | | |
| | - | 27.1 | ± 1 | | 1.9 | ± 0.1 | 1.3 | ± 0.0 | 54.9 | ± 1.4 | 27.8 | ± 1.7 |
| | 13 | 28.6 | ± 0.7 | | 1.4 | ± 0.1 | 1.6 | ± 0.0 | 55.1 | ± 1.7 | 26.5 | ± 1.8 |
| **Lova** | 25 | 34.0 | ± 0.9 ** | **Y-27632** | 1.3 | ± 0.1 | 0.9 | ± 0.0 *** | 58.8 | ± 1.8 | 24.7 | ± 2 |
| | 50 | 39.7 | ± 1 **** | | 2.2 | ± 0.3 | 0.9 | ± 0.0 *** | 61.2 | ± 1 | 21.5 | ± 1.4 |
| | | $F_{(3,8)}$=39.7 *P*=0.0001 | | | $F_{(3,8)}$=6 *P*=0.0191 | | $F_{(3,8)}$=22 *P*=0.0003 | | $F_{(3,8)}$=4. *P*=0.0493 | | $F_{(3,8)}$=2.5 *P*=0.1367 | |
| | - | 27.2 | ± 1.5 | | 15.3 | ± 1.8 | 2.3 | ± 0.5 | 38.3 | ± 0.7 | 11.1 | ± 1.7 |
| | 2.5 | 19.4 | ± 1 ** | | 15.9 | ± 1.5 | 2.3 | ± 0.4 | 33.0 | ± 1.2 * | 13.6 | ± 1.5 |
| **Wort** | 5 | 17.5 | ± 0.8 *** | **Lova** | 15.1 | ± 1.2 | 3.2 | ± 0.7 | 32.0 | ± 1.3 * | 14.4 | ± 1.5 |
| | 10 | 14.9 | ± 1.1 *** | | 17.1 | ± 1.9 | 1.9 | ± 0.3 | 30.7 | ± 1.2 ** | 15.8 | ± 1.6 |
| | | $F_{(3,8)}$=22 *P*=0.0003 | | | $F_{(3,8)}$=0.3 *P*=0.8196 | | $F_{(3,8)}$=1.1 *P*=0.4011 | | $F_{(3,8)}$=8.8 *P*=0.0065 | | $F_{(3,8)}$=1.6 *P*=0.2723 | |
| | - | 26.4 | ± 0.7 | | 3.0 | ± 0.2 | 1.1 | ± 0.1 | 56.6 | ± 1.7 | 30.2 | ± 1.9 |
| | 2.5 | 19.4 | ± 1 *** | | 2.4 | ± 0.2 | 1.0 | ± 0.1 | 49.7 | ± 1.8 * | 30.2 | ± 2.1 |
| **Wort** | 5 | 18.7 | ± 0.8 *** | **Y-27632** | 2.5 | ± 0.1 | 1.3 | ± 0.1 | 48.3 | ± 1.3 * | 29.5 | ± 1.5 |
| | 10 | 17.2 | ± 0.6 *** | | 3.2 | ± 0.2 | 1.1 | ± 0.1 | 48.4 | ± 1.4 * | 31.2 | ± 1.6 |
| | | $F_{(3,8)}$=26.8 *P*=0.0002 | | | $F_{(3,8)}$=4.6 *P*=0.0377 | | $F_{(3,8)}$=2 *P*=0.1893 | | $F_{(3,8)}$=6.4 *P*=0.0162 | | $F_{(3,8)}$=0.1 *P*=0.9252 | |
| **Secondary neurite length** | | | | | | | | | | | | |
| | - | 1.3 | ± 0.13 | | 8.0 | ± 0.5 | 1.3 | ± 0.1 | 11.8 | ± 0.64 | 10.5 | ± 0.66 |
| | 13 | 2.2 | ± 0.21 | | 5.0 | ± 0.5 * | 1.1 | ± 0.1 | 11.2 | ± 0.69 | 8.9 | ± 0.73 |
| **Lova** | 25 | 2.5 | ± 0.31 * | **Y-27632** | 4.2 | ± 0.5 ** | 0.9 | ± 0.1 | 13.3 | ± 0.77 | 10.7 | ± 0.83 |
| | 50 | 4.2 | ± 0.31 *** | | 5.7 | ± 0.7 | 1.0 | ± 0.1 | 15.4 | ± 0.8 * | 11.2 | ± 0.86 |
| | | $F_{(3,8)}$=22.6 *P*=0.0003 | | | $F_{(3,8)}$=8.6 *P*=0.0069 | | $F_{(3,8)}$=2.6 *P*=0.1257 | | $F_{(3,8)}$=6.6 *P*=0.0149 | | $F_{(3,8)}$=1.6 *P*=0.2698 | |
| | - | 1.3 | ± 0.15 | | 23.0 | ± 3.6 | 2.5 | ± 0.8 | 4.5 | ± 0.59 | 3.2 | ± 0.61 |
| | 2.5 | 0.6 | ± 0.13 ** | | 19.1 | ± 2.4 | 2.6 | ± 0.7 | 3.1 | ± 0.35 | 2.6 | ± 0.38 |
| **Wort** | 5 | 0.5 | ± 0.13 ** | **Lova** | 17.0 | ± 2.1 | 3.0 | ± 0.9 | 2.5 | ± 0.33 * | 2.1 | ± 0.35 |
| | 10 | 0.3 | ± 0.06 ** | | 19.1 | ± 2.4 | 2.0 | ± 0.4 | 2.7 | ± 0.15 * | 2.3 | ± 0.17 |
| | | $F_{(3,8)}$=12.2 *P*=0.0024 | | | $F_{(3,8)}$=0.9 *P*=0.4964 | | $F_{(3,8)}$=0.3 *P*=0.7902 | | $F_{(3,8)}$=5.2 *P*=0.0278 | | $F_{(3,8)}$=1.4 *P*=0.315 | |
| | - | 1.2 | ± 0.12 | | 5.6 | ± 0.4 | 1.4 | ± 0.1 | 12.0 | ± 1.12 | 10.8 | ± 1.13 |
| | 2.5 | 0.7 | ± 0.27 | | 6.1 | ± 0.4 | 1.6 | ± 0.1 | 8.5 | ± 0.72 | 7.8 | ± 0.77 |
| **Wort** | 5 | 0.6 | ± 0.1 | **Y-27632** | 6.0 | ± 0.4 | 1.5 | ± 0.1 | 8.2 | ± 0.55 * | 7.6 | ± 0.56 |
| | 10 | 0.6 | ± 0.04 | | 5.6 | ± 0.4 | 1.5 | ± 0.1 | 8.4 | ± 0.86 * | 7.8 | ± 0.87 |
| | | $F_{(3,8)}$=3.7 *P*=0.0617 | | | $F_{(3,8)}$=0.4 *P*=0.7357 | | $F_{(3,8)}$=0.4 *P*=0.7672 | | $F_{(3,8)}$=4.8 *P*=0.0337 | | $F_{(3,8)}$=3.2 *P*=0.0818 | |

Compounds were incubated 24 h in SH-SY5Y cells.
Results represent the means ± S.E.M. (n=3) and pharmacological parameters were determined by a nonlinear regression with a 4PL or normalized fitting. Statistical analysis were performed by one-way ANOVA followed by Bonferroni post hoc test: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs corresponding vehicle-treated cells (without 1st drug).
See figure 2 for respective curves.
Lova, Lovastatin. Wort, wortmannin. Δ, difference.

Table 10. (Supplemental Table 3) **Pharmacological parameters of the Y-27632, lovastatin and wortmannin dose-responses alone or in combination on the primary and secondary neurite number in SH-SY5Y cells**

| Number of the primary and secondary neurites : pharmacologic parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1st drug** (µM) | | **Basal value** without dose-response (neurite) | **2nd drug** Dose-response | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (neurite) | **$E_{max}$** (neurite number Δ over basal value) |
| **Primary neurite number/cell** | | | | | | | |
| | - | 2.23 ± 0.04 | | 1.1 ± 0.12 | 1.5 ± 0.2 | 3.06 ± 0.06 | 0.83 ± 0.07 |
| | 13 | 2.20 ± 0.03 | | 0.9 ± 0.12 | 1.1 ± 0.1 | 2.88 ± 0.04 | 0.68 ± 0.05 |
| **Lova** | 25 | 2.39 ± 0.07 | **Y-27632** | 0.9 ± 0.14 | 1.1 ± 0.1 | 3.03 ± 0.06 | 0.64 ± 0.09 |
| | 50 | 2.47 ± 0.04 * | | 1.4 ± 0.33 | 0.9 ± 0.1 | 2.98 ± 0.07 | 0.51 ± 0.08 |
| | | $F_{(3,8)}$=7.4 *P*=0.0108 | | $F_{(3,8)}$=1.7 *P*=0.2373 | $F_{(3,8)}$=2.6 *P*=0.1214 | $F_{(3,8)}$=1.8 *P*=0.2220 | $F_{(3,8)}$=3.2 *P*=0.0852 |
| | - | 2.29 ± 0.11 | | 9.6 ± 12.16 | 1.4 ± 1.5 | 2.38 ± 0.06 | 0.10 ± 0.13 |
| | 2.5 | 1.78 ± 0.06 * | | 12.2 ± 2.3 | 1.8 ± 0.5 | 2.28 ± 0.07 | 0.50 ± 0.08 * |
| **Wort** | 5 | 1.65 ± 0.05 ** | **Lova** | 11.2 ± 1.63 | 3.0 ± 1.1 | 2.16 ± 0.08 | 0.51 ± 0.07 * |
| | 10 | 1.47 ± 0.12 *** | | 9.6 ± 2.32 | 1.6 ± 0.5 | 2.06 ± 0.07 | 0.59 ± 0.07 * |
| | | $F_{(3,8)}$=15.2 *P*=0.0011 | | $F_{(3,8)}$=0.4 *P*=0.9883 | $F_{(3,8)}$=0.5 *P*=0.6663 | $F_{(3,8)}$=3.9 *P*=0.0539 | $F_{(3,8)}$=5.6 *P*=0.0222 |
| | - | 2.21 ± 0.05 | | 1.7 ± 0.44 | 1.2 ± 0.3 | 3.04 ± 0.11 | 0.83 ± 0.12 |
| | 2.5 | 1.73 ± 0.05 ** | | 1.3 ± 0.17 | 1.0 ± 0.1 | 2.69 ± 0.08 | 0.96 ± 0.09 |
| **Wort** | 5 | 1.78 ± 0.08 ** | **Y-27632** | 1.5 ± 0.24 | 1.3 ± 0.2 | 2.62 ± 0.1 | 0.84 ± 0.12 |
| | 10 | 1.68 ± 0.09 ** | | 1.2 ± 0.22 | 1.3 ± 0.2 | 2.57 ± 0.14 * | 0.89 ± 0.16 |
| | | $F_{(3,8)}$=12.1 *P*=0.0024 | | $F_{(3,8)}$=0.5 *P*=0.6714 | $F_{(3,8)}$=0.4 *P*=0.7684 | $F_{(3,8)}$=3.7 *P*=0.0598 | $F_{(3,8)}$=0.2 *P*=0.8756 |
| **Secondary neurite number/primary neurite** | | | | | | | |
| | - | 0.062 ± 0.003 | | 9.8 ± 0.57 | 1.3 ± 0.1 | 0.371 ± 0.018 | 0.310 ± 0.018 |
| | 13 | 0.098 ± 0.009 * | | 6.6 ± 0.62 ** | 1.0 ± 0.1 | 0.339 ± 0.012 | 0.240 ± 0.015 * |
| **Lova** | 25 | 0.101 ± 0.009 * | **Y-27632** | 5.0 ± 0.45 *** | 1.0 ± 0.1 | 0.396 ± 0.01 | 0.295 ± 0.014 |
| | 50 | 0.141 ± 0.009 *** | | 5.0 ± 0.51 *** | 1.0 ± 0.1 | 0.447 ± 0.013 * | 0.306 ± 0.016 |
| | | $F_{(3,8)}$=16.6 *P*=0.0009 | | $F_{(3,8)}$=17.1 *P*=0.0008 | $F_{(3,8)}$=3.5 *P*=0.0696 | $F_{(3,8)}$=11.3 *P*=0.0030 | $F_{(3,8)}$=4.2 *P*=0.0462 |
| | - | 0.060 ± 0.005 | | 22.0 ± 3.48 | 2.5 ± 0.8 | 0.155 ± 0.016 | 0.095 ± 0.017 |
| | 2.5 | 0.035 ± 0.008 | | 17.9 ± 2.21 | 2.4 ± 0.6 | 0.127 ± 0.01 | 0.092 ± 0.013 |
| **Wort** | 5 | 0.032 ± 0.007 * | **Lova** | 19.1 ± 2.15 | 3.1 ± 0.8 | 0.115 ± 0.012 | 0.083 ± 0.014 |
| | 10 | 0.030 ± 0.004 * | | 16.7 ± 2.6 | 2.1 ± 0.6 | 0.107 ± 0.006 | 0.077 ± 0.007 |
| | | $F_{(3,8)}$=5.1 *P*=0.0294 | | $F_{(3,8)}$=0.7 *P*=0.5627 | $F_{(3,8)}$=0.3 *P*=0.8292 | $F_{(3,8)}$=3.3 *P*=0.0789 | $F_{(3,8)}$=0.4 *P*=0.7647 |
| | - | 0.061 ± 0.007 | | 12.1 ± 1.17 | 1.5 ± 0.2 | 0.323 ± 0.027 | 0.026 ± 0.013 |
| | 2.5 | 0.039 ± 0.015 | | 8.3 ± 0.67 * | 1.3 ± 0.1 | 0.267 ± 0.022 | 0.031 ± 0.017 |
| **Wort** | 5 | 0.033 ± 0.007 | **Y-27632** | 6.4 ± 0.65 ** | 1.5 ± 0.2 | 0.218 ± 0.021 * | 0.021 ± 0.008 |
| | 10 | 0.038 ± 0.009 | | 7.3 ± 0.96 * | 1.3 ± 0.2 | 0.201 ± 0.015 * | 0.022 ± 0.013 |
| | | $F_{(3,8)}$=1.5 *P*=0.2790 | | $F_{(3,8)}$=7.9 *P*=0.087 | $F_{(3,8)}$=0.4 *P*=0.7672 | $F_{(3,8)}$=6.4 *P*=0.0161 | $F_{(3,8)}$=0.1 *P*=0.9460 |

Compounds were incubated 24 h in SH-SY5Y cells.
Results represent the means ± S.E.M. (n=3) and pharmacological parameters were determined by a nonlinear regression with a 4PL or normalized fitting. Statistical analysis were performed by one-way ANOVA followed by Bonferroni post hoc test: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs corresponding vehicle-treated cells (without 1st drug).
See figure 2 for respective curves.
Lova, lovastatin; Wort, wortmannin; Δ, difference.

Table 11. (Supplemental Table 4) **Lovastatin treatment reduces the [$^3$H]dopamine uptake in SH-SY5Y cells**

| Timing incubation | Lovastatin | | 24 h | | 48 h | |
|---|---|---|---|---|---|---|
| | DA uptake | | 15 min | 60 min | 15 min | 60 min |
| $V_{max}$ (pmol/mg protein/min) | Vehicle | - | 10.5 ± 0.31 | 7.2 ± 0.29 | 8.2 ± 0.45 | 5.9 ± 0.2 |
| | Lova (µM) | 5 | 11.8 ± 0.50 | 6.8 ± 0.32 | 7.9 ± 0.88 | 3.9 ± 0.14 * |
| | | 10 | 11.8 ± 0.90 | 6.5 ± 0.37 | 6.5 ± 0.45 | 3.6 ± 0.27 ** |
| | | 50 | 7.2 ± 0.49 * | 4.5 ± 0.21 *** | 5.4 ± 0.81 | 2.5 ± 0.36 *** |
| | | | $F_{(3,8)}$=12.91, $P$<0.002 | $F_{(3,8)}$=16.06, $P$<0.001 | | $F_{(3,8)}$=34.77, $P$<0.0001 |
| $K_m$ (nM) | Vehicle | - | 277 ± 30 | 188 ± 30 | 240 ± 49 | 203 ± 26 |
| | Lova (µM) | 5 | 297 ± 49 | 209 ± 38 | 344 ± 129 | 198 ± 27 |
| | | 10 | 320 ± 84 | 204 ± 45 | 289 ± 74 | 240 ± 56 |
| | | 50 | 212 ± 56 | 160 ± 31 | 490 ± 231 | 339 ± 165 |

Michaelis-Menten fitting equation was applied for calculations of kinetics parameters. $K_m$: substrate concentration that gives half maximal rate of DA transport (nM). $V_{max}$: maximal rate of DA transport (pmol/mg of protein/min). DA uptake saturation concentration range: 15 nM to 3 µM.
Results represent the means ± S.E.M. (n = 3) and significant differences were measured by one-way ANOVA followed by Bonferroni post hoc test; *$P$<0.05, **$P$<0.01, ***$P$<0.001 for comparison between vehicle and treated cells were represented in respective column. Lova: lovastatin. DA: dopamine
See figure 6 for respective curves

Table 12. (Supplemental Table 5) **Pharmacological inhibition parameters of GBR-12935, RSP and TBZ dose-response treatments on [$^3$H]dopamine uptake in SH-SY5Y cells treated with lovastatin and in BE(2)-M17 cells transfected with VMAT2**

| SH-SY5Y cells: [$^3$H]DA uptake: pharmacologic parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lovastatin | | | Dose-response | $IC_{50}$ | | | $n_H$ | | $I_{max}$ (%) | | |
| Incubation time | | (µM) | | | | | | | | | |
| 24 h | - | - | GBR-12935 ($IC_{50}$: nM) | 424 | ± 50 | | -1.9 | ± 0.3 | 100.0 | ± 0.8 | |
| | + | 50 | | 348 | ± 77 | | -1.9 | ± 0.7 | 99.0 | ± 4.8 | |
| | - | - | RSP ($IC_{50}$: pM) | 916 | ± 140 | | -0.9 | ± 0.1 | 85.4 | ± 3.2 | |
| | + | 50 | | 584 | ± 64 | | -0.9 | ± 0.1 | 77.5 | ± 2.2 | |
| | - | - | TBZ ($IC_{50}$: µM) | 20.4 | ± 5.6 | | -0.7 | ± 0.1 | 74.9 | ± 3.4 | |
| | + | 50 | | 26.6 | ± 19.4 | | -0.4 | ± 0.1 | 73.0 | ± 5.2 | |
| | + | 50 | High affinity site ($IC_{50}$: nM) | 62.9 | ± 278 | | | | | | |
| 48 h | - | - | GBR-12935 ($IC_{50}$: nM) | 252 | ± 8.4 | | -2.0 | ± 0.1 | 99.7 | ± 1.2 | |
| | + | 5 | | 189 | ± 7.9 | ** | -1.9 | ± 0.1 | 99.1 | ± 1.2 | |
| | | 10 | | 181 | ± 11.8 | *** | -1.9 | ± 0.2 | 99.3 | ± 2.3 | |
| | | 50 | | 170 | ± 11.8 | ** | -1.9 | ± 0.2 | 99.4 | ± 2.3 | |
| | - | - | RSP ($IC_{50}$: pM) | 182 | ± 15.6 | | -1.5 | ± 0.2 | 77.9 | ± 2.1 | |
| | + | 5 | | 150 | ± 24.6 | | -1.2 | ± 0.2 | 59.7 | ± 2.7 | ** |
| | | 10 | | 199 | ± 34.3 | | -1.3 | ± 0.2 | 50.2 | ± 2.5 | **** |
| | | 50 | | 699 | ± 192 | ** | -1.1 | ± 0.3 | 36.7 | ± 3.7 | **** |
| | - | - | TBZ ($IC_{50}$: µM) | 4.6 | ± 0.9 | | -0.6 | ± 0.1 | 77.9 | ± 3.2 | |
| | + | 5 | | 2.6 | ± 0.57 | | -0.5 | ± 0.0 | 73.0 | ± 10.1 | |
| | | 10 | | 5.4 | ± 1.25 | | -0.6 | ± 0.1 | 61.4 | ± 3.3 | |
| | | 50 | | 10.5 | ± 4.37 | | -0.8 | ± 0.2 | 48.1 | ± 4.7 | * |

| BE(2)-M17 cell : [$^3$H]DA uptake: pharmacologic parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Transfection & affinity site | | | Dose-response | $IC_{50}$ | | | $n_H$ | | $I_{max}$ (%) | | |
| WT | | | RSP ($IC_{50}$: pM) | 1.53 | ± 0.23 | | -1.2 | ± 0.4 | 53.1 | ± 2.1 | |
| VMAT2 | | | | 5.25 | ± 0.57 | *** | -1.2 | ± 0.5 | 78.7 | ± 3 | *** |
| WT | Low affinity | | TBZ (Affinity $IC_{50}$: low in µM and high in nM) | 25.8 | ± 11.8 | | -0.8 | ± 0.2 | 65.7 | ± 3.8 | |
| | High affinity | | | 71.9 | ± 10.6 | | | | | | |
| VMAT2 | Low affinity | | | 18.8 | ± 12.3 | | -0.3 | ± 0.0 | 82.1 | ± 2.9 | ** |
| | High affinity | | | 27 | ± 11.7 | * | | | | | |

[$^3$H]DA concentration: 15 nM for 60 min. GBR-12935, RSP and TBZ were incubated 30 min prior the DA uptake. -, no co-treatment. +, co-treatment.

Results represent the means ± S.E.M. (n=3-5) and pharmacological parameters were determined by a nonlinear regression with a 4PL or normalized fitting.

SH-SY5Y: statistical analysis were performed by one-way ANOVA in SH-SY5Y cells ($IC_{50}$: GBR-12935 and lovastatin 48h: $F_{(3,12)=}13.43$, $P$=0.0004; $IC_{50}$: RSP and lovastatin 48h: $F_{(3,12)=}7.06$, $P$=0.0054; $I_{max}$: RSP and lovastatin 48h: $F_{(3,12)=}37.29$, $P$<0.0001; $I_{max}$: TBZ and lovastatin 48h $F_{(3,12)=}4.850$, $P$=0.0195) followed by Bonferroni post hoc test; *$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001 for comparison between vehicle and lovastatin treatment.

Be(2)-M17: statistical analysis were performed by a student's $t$-test for comparison between wild-type and VMAT2 transfection in Be(2)-M17 ($IC_{50}$ RSP: $t_{(8)}$=6.05, $P$=0.0003 n=5; $IC_{50}$ TBZ high affinity site: $t_{(8)}$=15.78, $P$=0.0216 n=5; TBZ $n_H$: $t_{(8)}$=2.50, $P$=0.0365 n=5; RSP $I_{max}$: $t_{(8)}$=6.99, $P$=0.0001 n=5; TBZ $I_{max}$: $t_{(8)}$=3.42, $P$=0.0089 n=5).

See figure 6 g-l and figure 7 c-d for respective curves.

DA, dopamine; RSP, reserpine; TBZ, tetrabenazine; VMAT2, transfection with VMAT2; WT, wild-type.

# V. Sterol regulatory element-binding protein-1 (SREBP-1) activation modulates dopaminergic phenotype of neuroblastoma cells

## V. 1. Abstract

Statins may reduce the risk of Parkinson's disease (PD) suggesting a neuroprotective role to counteract the degeneration of dopaminergic neurons. Several cellular signalling cascades are modulated by the statin-mediated inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The transcriptional activation of the sterol regulatory elementary binding protein (SREBP) is one of the major cellular signalling pathways mediated by cholesterol-lowering effect of statins. We previously demonstrated that statins induce an up-regulation and functional changes of presynaptic dopaminergic proteins in neuroblastoma cells and hypothesized that SREBP activation could mediate these effects, leading to decreased incidence of PD amongst statin users. Here, we test this hypothesis, reporting that the SREBP activator U18666A, increases the translocation of SREBP-1 into the nucleus, inducing an increase in presynaptic dopaminergic marker expression such as vesicular monoamine transporter 2 (VMAT2), synaptic vesicle glycoprotein 2A (SV2A) and 2C, synaptogyrin-3 (SYNGR3) and tyrosine hydroxylase. siRNA-mediated SREBP-1 downregulation also abolishes the expression of VMAT2 and SV2C. Our results thus show that U18666A induces cell differentiation, through SREBP-1 activation, towards dopaminergic phenotype, suggesting that SREBP-1 transcriptional activity may mediate, at least in part, the neuroprotective effects observed following statins treatment in PD, shedding light upon SREBP-1 as a potential new target for developing disease-modifying treatment in PD.

# V. 2. Introduction

Epidemiological studies show an inverse association between the use of statins and the incidence of Parkinson's disease (PD) (Wolozin et al., 2007; Wahner et al., 2008; Gao et al., 2012; Lee et al., 2013) suggesting an underlying neuroprotective mechanism of statins. Such potential effect has been then confirmed in experimental PD models using neurotoxic agents such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Ghosh et al., 2009; Castro et al., 2013) and 6-hydroxydopamine (Yan et al., 2011; Xu et al., 2013). The protection is thought to occur through their action against proinflammatory processes, oxidative stress and α-synuclein aggregation (Ghosh et al., 2009; Roy and Pahan, 2011; Kumar et al., 2012; Xu et al., 2013), via the PI3K/Akt and the Ras homolog gene family member A (RhoA) pathways (Schulz et al., 2004; Evangelopoulos et al., 2009; Racchetti et al., 2010; Jin et al., 2012; Raina et al., 2013). We recently proposed an alternate mechanism by which statins induce differentiation of neuroblastoma SH-SY5Y cells towards a dopaminergic phenotype (Schmitt et al., 2015), i.e. statins would oppose ongoing degeneration in PD. Statins indeed up-regulate the expression of the presynaptic dopaminergic markers cells such as vesicular monoamine transporter 2 (VMAT2), synaptogyrin 3 (SYNGR3) or synaptic vesicles glycoprotein 2C (SV2C) that are the key players of the intravesicular dopamine regulation (Schmitt et al., 2015). Promoting uptake by vesicles may reduce the toxicity of oxidized dopamine and of neurotoxic agents (Egana et al., 2009; Dardou et al., 2013; Segura-Aguilar et al., 2014).

We hypothesized that sterol regulatory element binding proteins-1 (SREBP-1) activation, mediated by cholesterol-lowering effect of statins, could mediate these effects, leading to decreased incidence of PD amongst statin users. SREBPs are transcription factors responsible for the upregulation of enzymes involved in the lipid and cholesterol synthesis synthesis (Kim and Spiegelman, 1996; Shimano et al., 1999; Eberlé et al., 2004; Espenshade, 2006; Jeon and Osborne, 2012; Shao and Espenshade, 2014). Under conditions of sufficient cholesterol levels, the cholesterol sensor of SREBP cleavage activating protein (SCAP) prevents the activation of SREBP (Yabe et al., 2002; Yang et al., 2002; Eberlé et al., 2004; Engelking et al., 2005; Espenshade, 2006; Jeon and Osborne, 2012). When the cholesterol is low, e.g. under statin exposure, SREBP is released and cleaved by the site-1 protease (S1P) and site-2-zing metalloproteinase in a truncated active form. This SREBP proteolytic activation allows its translocation into the nucleus where it leads to a transcriptional

activation of lipid-biosynthesis enzymes (Eberlé et al., 2004; Espenshade, 2006; Jeon and Osborne, 2012) and probably the synaptic markers.

However, the direct link between SREBP-1 activation and the induction of dopaminergic marker expression remains to be demonstrated and characterized. To this end, we investigated the role of SREBP in the modulation of the dopaminergic phenotype (at protein and mRNA levels) in neuroblastoma cells using genetic and pharmacologic modulators of SREBP.

# V. 3. Materials and methods

## I. 1.1. Materials

SH-SY5Y (CRL-2266) and BE(2)-M17 (95011816) cells were obtained from ATCC (American Type Culture Collection, Molsheim, France) and ECACC (European Collection of Cell Cultures, Salisbury, UK), respectively. Reagents for cell culture were purchased to Lonza (Verviers, Belgium). Coating reagent poly-D-lysine and collagen were from Sigma-Aldrich (Diegem, Belgium) and Corning (Lasne, Belgium) respectively. Primary antibodies were βIII-tubulin (MMS-435P or PRB-435P) from Covance (Rotterdam, Netherlands), tyrosine hydroxylase (AB152) from Millipore (Overijse, Belgium), SYNGR3 (sc-271046) from Santa Cruz Biotechnology (Dallas, TX, USA), SREBP-1 (SAB4502850), β-actin (A3853 and A2066) from Sigma-Aldrich, synaptic vesicle glycoprotein 2A (119 002), SV2C (119 202) and VMAT2 (138 302) from Synaptic Systems (Goettingen, Germany). Secondary antibodies were from Life Technologies (Alexa Fluor-488 and 647-conjugated goat anti-mouse and/or anti-rabbit IgG; Gent, Belgium). DAPI, lipofectamine RNAiMAX, high capacity cDNA reverse transcription kit with MultiScribe MuLV transcriptase, Luminaris probe qPCR master mix (low ROX), Taqman PCR probe (FAM-MGB) and serum for cell culture were from Life Technologies. U18666A and PF-429242 were purchased to Tocris (Abingdon, UK). Mock and SREBP-1 siRNAs were from GE Healthcare Dharmacon (Diegem, Belgium).

## I. 1.2. Cell cultures and drug treatments

SH-SY5Y and BE(2)-M17 neuroblastoma cells were cultured in Dulbecco's modified Eagle medium (DMEM) medium supplemented with 10% (v/v) of foetal bovine serum and maintained in a humidified incubator with 95% air and 5% $CO^2$ at 37 °C. For immunocytochemistry, the cells were seeded (15 x $10^3$) 24 h before the studies on poly-D-lysine and collagen mix pre-coated 96-well plates. For gene expression studies, cells were plated in 48-well plates at 160 x $10^3$ cells per well per well for SH-SY5Y cells. For siRNA study, BE(2)-M17 cells were plated in 48-well at 50 x $10^3$ cells. The cells were stimulated with pharmacological agents by incubation with single or multiple increasing doses.

### I. 1.3. Immunocytochemistry and high-content image analysis

The Immunocytochemistry and high-content image analysis protocol was previously described (Schmitt et al., 2015).The effects of pharmacological treatments on SREBP-1 nuclear translocation and protein expression were analyzed by immunofluorescence method using antibodies against the synaptic markers and SREBP-1. Briefly, cells were washed with phosphate-buffered saline (PBS) followed by fixation with 4% paraformaldehyde for 30 min (room temperature) and permeabilization (triton X-100 0.05% -10 min). The non-specific binding was blocked with blocking solution (3% of bovine serum albumin and 5% of normal goat serum in PBS) for 1 h. The cells were incubated overnight at 4 °C with primary antibodies against SV2A (1:500), SV2C (1:500), VMAT2 (1:500), SREBP-1 (1:500), SYNGR-3 (1:100) or βIII-tubulin (1:3000) in blocking solution. After washing (4 x 5 min), the cells were incubated with species-specific corresponding secondary antibodies labelled with Alexa Fluor 488 or 647 in blocking solution. Nuclear counterstaining was performed by incubation with DAPI and by further washing steps (5 x 5 min).

Immunofluorescence signal was analysed using a high-content imaging (HCI) microscopy system (BD Pathway-855 Bioimager System using BD Attovision, Becton-Dickinson, Erembodegem, Belgium) in PBS medium. Series (2 by 3) of images were acquired in 3 non-superposing image fields per well using a 20x objective (0.75NA, Olympus, Berchem, Belgium). The fluorescent intensity of neuronal markers and nuclear translocation were analysed using a specific image analysis algorithms developed using Cellenger software package (Definiens, München, Germany).. Cytoplasmic and nuclear intensity parameters were used for determination of protein levels and nuclear translocation. Both quantifications use the cell compartmentalization defined by segmentation of βIII-tubulin staining for the cytoplasm and DAPI for the nucleus. For quantification of protein levels, the relative intensity of cytoplasm was measured minus the background value. The nuclear translocation was measure by quantification of the nuclear/cytoplasmic intensity ratio.

### I. 1.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

The qRT-PCR protocol was previously described (Schmitt et al., 2015).Total cellular RNA was isolated using the RNeasy mini kit (Qiagen, Venlo, Netherlands) according to the

manufacturer's protocol. Briefly, cDNA was synthetized from 1.5 µg of total RNA using high capacity cDNA reverse transcription kit (20 µl). The quantitative real-time PCR experiments were performed in MicroAmp- 96-wellPCR plates using specific Taqman gene expression probes for *SV2A* (Hs00372069_m1), *SV2C* (Hs00392676_m1), *SREBP1* (Hs01088691_m1), *SREBP2* (Hs01081784_m1), *SYNGR3* (Hs00188379_m1), *VMAT2* (*SLC18A2*; Hs00996835_m1), *TH* (Hs00165941_m1) and *β-actin* (*ACTB*; Hs99999903_m1). The reactions were performed in ViiA™ 7 RT-PCR system (Applied Biosystems) using 25 ng of cDNA sample and the recommended concentration of the specific probe and qPCR master mix Luminaris. PCR reactions were run in triplicate and the fold-changes in mRNA levels were calculated using $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) and normalized to β-actin mRNA levels.

### I. 1.5. SREBP-1 SiRNA transfection

For inducing SREBP-1 downregulation, cells were transfected with SREBP-1 siRNA On-TARGETplus SMARTpool (L-006891-00, *human SREBF1*, 4 target sequences: GCAACACAGCAACCAGAAA – CGGAGAAGCUGCCUAUCAA – GAAUAAAUCUGCUGUCUUG – GCGCACUGCUGUCCACAAAA) SiRNA 25 nM final and with non-targeting control pool (Mock, D-001810-10-05, 4 target sequences: UGGUUUACAUGUCGACUAA – UGGUUUACAUGUUGUGUGA – UGGUUUACAUGUUUUCUGA – UGGUUUACAUGUUUUCCUA) prepared in Lipofectamine RNAiMAX (2 µg/ml). Plasmid solution and Lipofectamine RNAiMAX were pre-diluted separately for 5 min in serum free medium and mixed for 30 min before added into the well. Cells were used 72 h after transfection.

### I. 1.6. Data analysis

Results are presented as means ± S.E.M. from a minimum of three independent experiments in duplicate or triplicate unless otherwise stated. Data were analysed with GraphPad Prism software (La Jolla, CA, USA) using either Student's t-test or ANOVA with Bonferroni's post hoc test. $P<0.05$ was considered significant to assess the difference between conditions. Half maximal effective concentration ($EC_{50}$) or inhibitory concentration ($IC_{50}$), maximal effect ($E_{max}$) or inhibition ($I_{max}$), Hill coefficient ($n_H$) and basal level pharmacological parameters were generated with nonlinear regression and fitted to four-parameter logistic curve (4PL);

$$Y = basal\ level + \frac{maximal\ effect - basal\ level}{1 + 10^{(LogEC_{50}\ or\ IC_{50} - X)n_H}}$$

and normalized equation;

$$Y = \frac{100}{1 + 10^{(LogEC_{50}\ or\ IC_{50} - X)n_H}}$$

# V. 4. Results

## V. 4.1. U18666A-induced translocation of SREBP-1 into nucleus in SH-SY5Y cells

We investigate the effects of U-18666A on the nuclear translocation of SREBP-1. U18666A is an known activator of SREBP in non-neuronal cells (Lange et al., 1999; Worgall et al., 2002; Zhang et al., 2004; Colgan et al., 2007). We first validated its ability to induce SREBP translocation in neuronal SH-SY5Y cells. Using SREBP-1 immunocytochemistry (Figure 1A), we studied the impact of U18666A upon the nuclear translocation of transcription factor. High-content analysis demonstrated a significant increase in U18666A–induced SREBP-1 translocation (Figure 1A) in a dose- and time-dependent manner in SH-SY5Y cells after 12 h of incubation (Figure 1B and Supplementary Table S1). A maximal effect was observed after 48 h of incubation with an $EC_{50}$ of 7±0.5 µM (Figure 1B and Supplementary Table S1). We then assessed the impact of PF-429242, which is thought to inhibit the SREBP cleavage activity of S1P enzyme (Hay et al., 2007; Hawkins et al., 2008) leading to an impairment of translocation (Hay et al., 2007; Hawkins et al., 2008; Olmstead et al., 2012; Shao and Espenshade, 2012). In our conditions, PF-429242 showed only a partial effect on SREBP-1 translocation at 48 h of incubation (Figure 1A, C and Supplementary Table 1S), culminating at 30±4.3% of the activation effect with U18666A treatment (48h, 30 µM, *t*-test $t_{(4)}$=10.5, $P$=0.0006). Overall, these data confirm that U18666A induces a significant and fast translocation of SREBP-1 while PF-429242 achieves 30% of this effect offering the possibility to investigate impact of different intensities of translocation upon induction of dopaminergic phenotype in neuroblastoma cells.

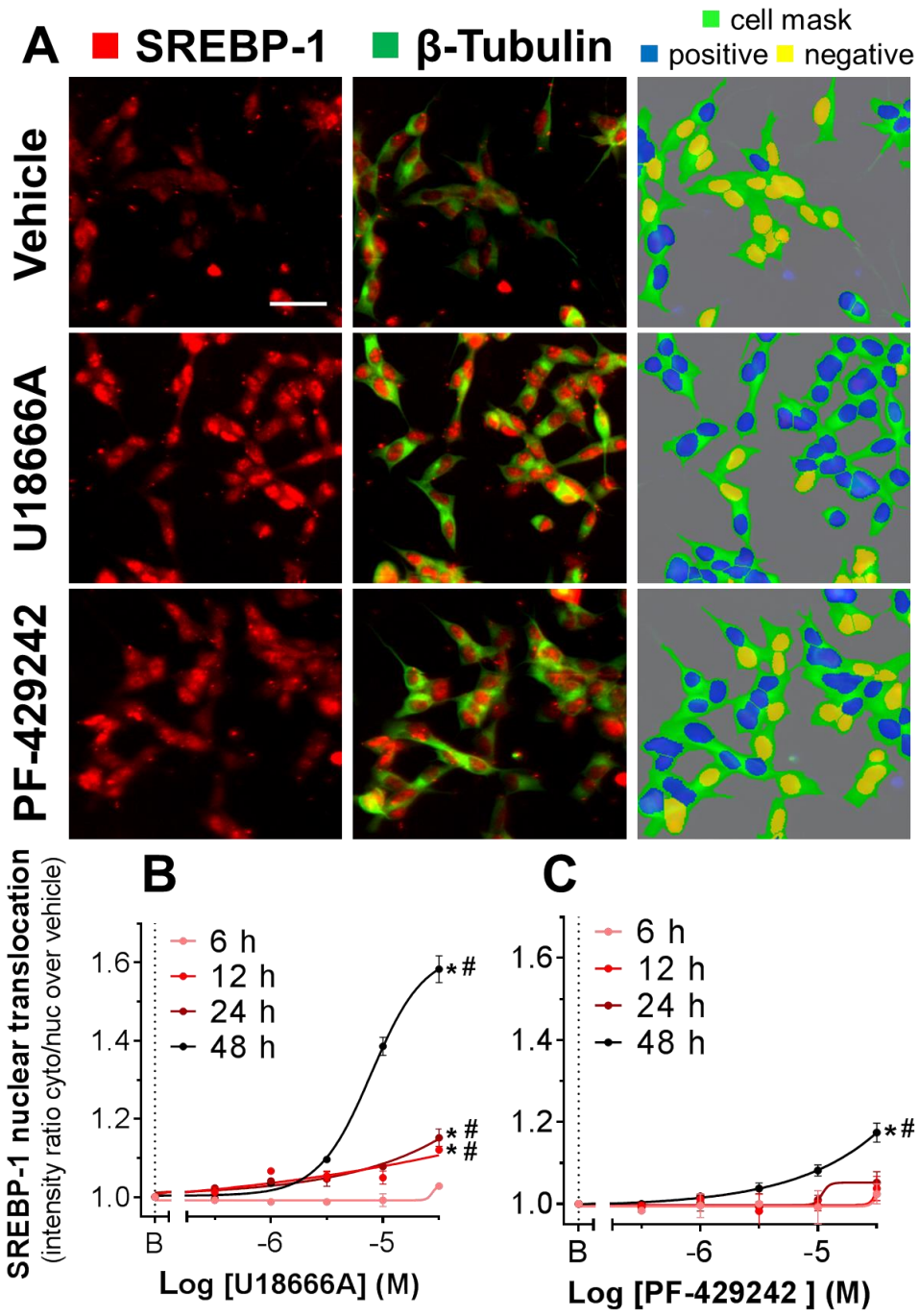


Figure 23. (Figure 1.) **U18666A triggers nuclear translocation of SREBP-1 in SH-SY5Y cells**

(**A**) Representative immunofluorescence images of SREBP-1 and β-tubulin staining in SH-SY5Y treated with 10 µM of U18666A and PF-429242. Classification of cells as positive or negative for the SREBP-1 nuclear translocation. (**B-C**) Dose-response kinetics (6 to 48 h) of (**B**) U18666A and (**C**) PF-429242 treatments on SREBP-1 nuclear translocation. Results represent the mean ± S.E.M. (n=4) and were fitted by nonlinear regression with a 4PL equation. Statistical analyses were performed by two-way ANOVA (U18666A: dose-response effect: $F_{(5,24)}$=192.6, $P$<0.0001; Time: $F_{(3,24)}$=239.8, $P$<0.0001; Interaction: $F_{(15,24)}$=76.43, $P$<0.0001; PF-429242 dose-response effect: $F_{(5,48)}$=10.06, $P$<0.0001; Time: $F_{(3,48)}$=13.56, $P$<0.0001; Interaction: $F_{(15,48)}$=2.742, $P$<0.0001) followed by Bonferroni post hoc test: */# were represented when a point of the curve was at least $P$<0.05 compared to baseline (*) or 6 h of treatment (#). Scale bar indicates 20 µM. B, basal level.

## V. 4.2. Activation of SREBP-1 translocation upregulates the presynaptic dopaminergic-related proteins

The activation of SREBP-1 translocation induced by U18666A may positively changes the transcriptional expression of neuronal markers. Using both gene expression analysis and immunofluorescence, we thus investigated the mRNA expression levels of several key proteins of dopaminergic system previously examined in the statin-induced dopaminergic differentiation study. Quantitative PCR using mRNA-specific probes showed that SREBP-1 and SREBP-2 mRNA expression levels were expectedly enhanced from 6 h of incubation with U18666A while they were inhibited after incubation with PF-429242 (Figure 2A and 2B). U18666A-induced increase in SV2A mRNA (Figure 2C) reached maximal effect at 24 h and 48 h of incubation in SH-SY5Y cells while 24 h of incubation induced a significant increase in VMAT2 mRNA that continuously increased up to 48 h after treatment (Figure 2E). A significant higher transcription level of SYNGR3 mRNA was observed after 6 h of incubation with U18666A and the expression level remained stable over time up to 48 h (Figure 2F). However, no detectable changes were found for mRNA levels of SV2C and TH (Figure 2D and G). Of note, PF-429242 did not modify mRNA levels for any of the studied synaptic proteins.

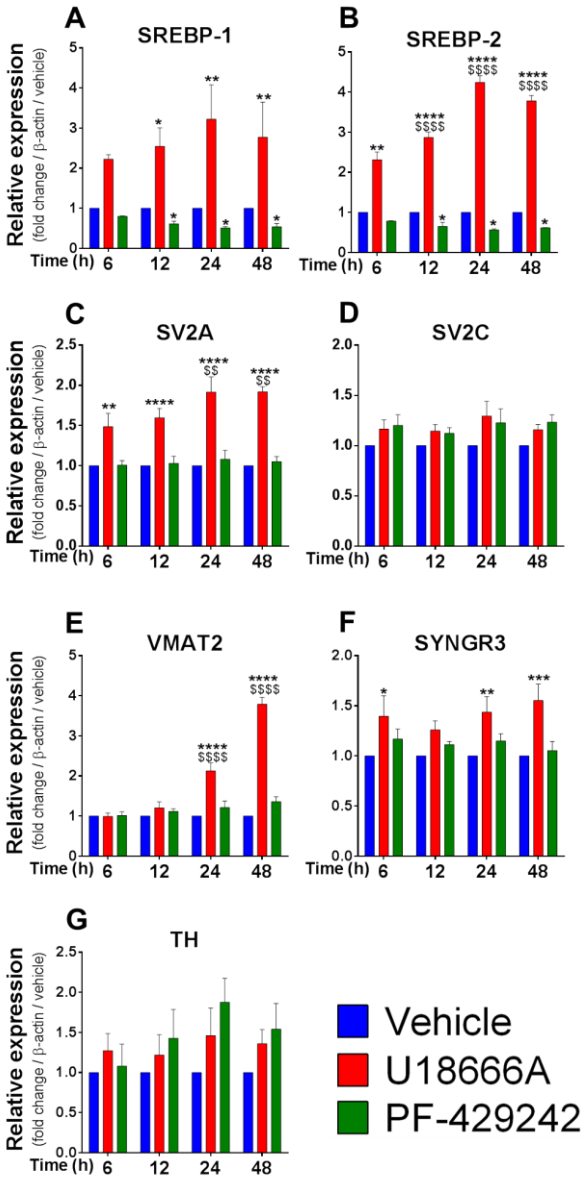


Figure 24. (Figure 2.) **U18666A treatment up-regulates mRNA levels of dopaminergic synaptic proteins**

(**A -G**) Time-course study (6-48 h) of (**A**) SV2A, (**B**) SV2C, (**C**) VMAT2, (**D**) SYNGR3, (**E**) TH, (**F**) SREBP-1 and (**G**) SREBP-2 mRNA expressions normalized over β-actin in SH-SY5Y cells treated with 10µM of U18666A and PF-429242 Results represent means ± S.E.M. (n=3). Statistical analyses were performed by two-way ANOVA (SV2A: treatment effect: $F_{(2,36)}$=74.76, $P$<0.0001; Time: $F_{(3,36)}$=2.341, $P$=0.0895; Interaction: $F_{(9,53)}$=1.633, $P$=0.1663. SV2C: treatment effect: $F_{(2,36)}$=7.971, $P$=0.0014; Time: $F_{(3,36)}$=0.5808, $P$=0.6315; Interaction: $F_{(6,36)}$=0.3024, $P$=0.9316.VMAT2: treatment effect: $F_{(2,36)}$=93.82, $P$<0.0001; Time : $F_{(3,36)}$=51.88, $P$<0.0001; Interaction: $F_{(6,36)}$=37.82, $P$<0.0001. SYNGR3: treatment effect: $F_{(2,36)}$=17.13, $P$<0.0001; Time: $F_{(3,36)}$=0.366, $P$=0.7774; Interaction: $F_{(6,36)}$=0.632, $P$=0.7034. TH: treatment effect: $F_{(2,36)}$=4.457, $P$=0.0186; Time: $F_{(3,36)}$=1.066, $P$=0.3757; Interaction: $F_{(6,36)}$=0.5608, $P$=0.7584. SREBP-1: treatment effect: $F_{(2,12)}$=33.72, $P$<0.0001; Time: $F_{(3,36)}$=0.2187, $P$=0.8815; Interaction: $F_{(6,12)}$=0.5545, $P$=0.7582. SREBP-2: treatment effect: $F_{(2,12)}$=888, $P$<0.0001; Time: $F_{(3,12)}$=22.04, $P$<0.0001; Interaction: $F_{(6,12)}$=30.16, $P$<0.0001) followed by Bonferroni post hoc test: ;*/#P<0.05, **/##P<0.01, ***/###P<0.001, ****/####P<0.0001, * vs corresponding vehicle treated cells, # vs corresponding 6 h of treatment. B, basal level.

We then determined if U18666A-induced increased transcriptional activity is accompanied by an increased mRNA translation. High content image analysis demonstrated that U18666A induced a dose-dependent increase in synaptic protein levels after 48 h of treatment (Figure 3A-E and Supplementary Table S2). The maximum increase of fluorescence intensity was observed by U18666A with a rank order among studied markers such as SV2C>SV2A=VMAT2=TH>SYNGR3. The potency of U18666A corresponds to a general $EC_{50}$ of 9.7±1 µM (Figure 3A-E and Supplemental Table S2), whereas no significant effect on synaptic protein level was observed for PF-429242 treatment (Figure 3A-E and Supplemental Table S2) as expected from the transcriptional results.

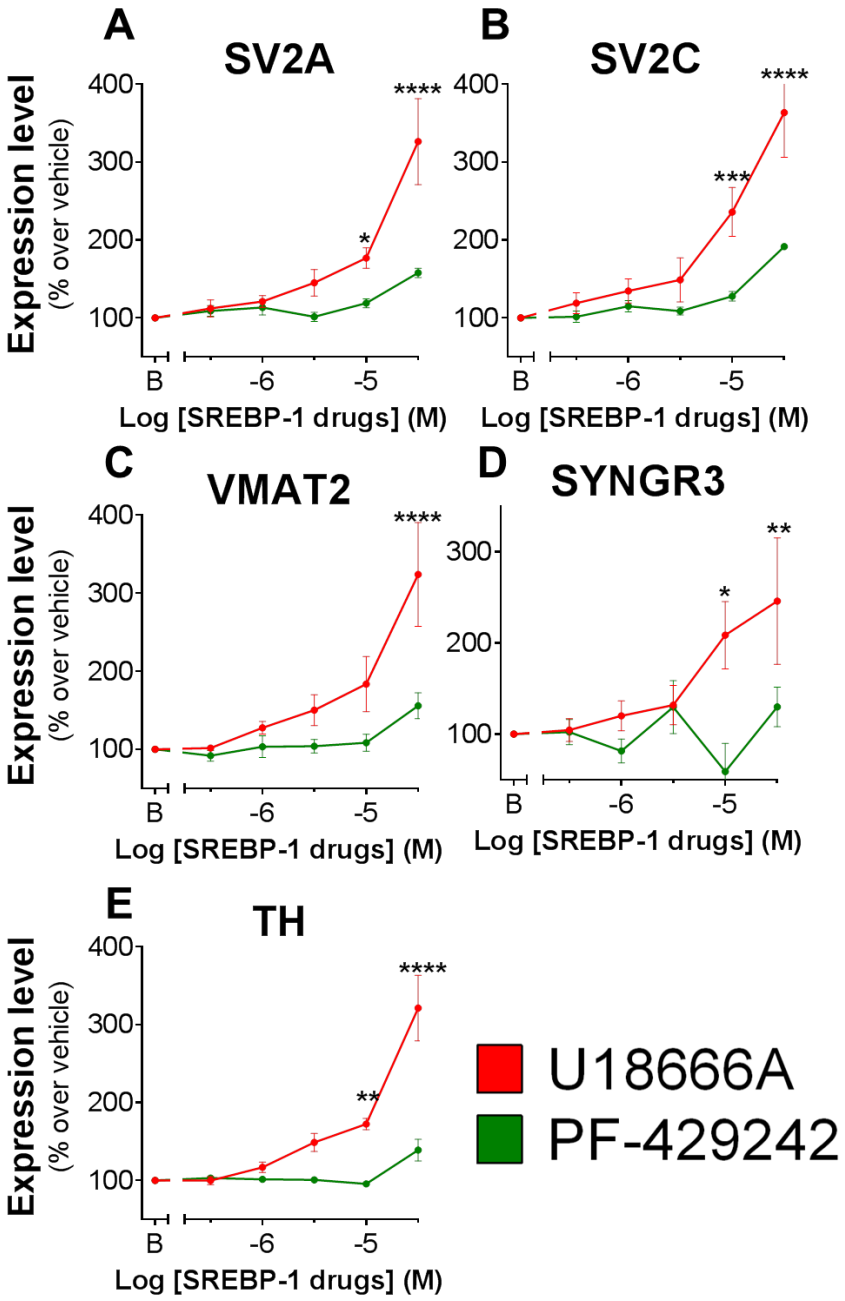


Figure 25. (Figure 3.) **Dopamine-related synaptic markers are increased by the U18666A treatment in SH-SY5Y cells**

(**A-F**) Quantification of (**A**) SV2A, (**B**) SV2C, (**C**) VMAT2, (**D**) SYNGR3 and (**E**) TH expression levels in cells treated for 24 h or 48 h with U18666A and PF-429242. Results represent means ± S.E.M. (n=3). Significant differences were measured by two-way ANOVA (SV2A: dose-response effect: $F_{(5,24)}$=16.77, *P*<0.0001; Compounds effect: $F1_{(,24)}$=20.06, *P*=0.0002; Interaction: $F_{(5,24)}$=6.274, *P*=0.0007. SV2C: dose-response effect: $F_{(5,24)}$=18.97, *P*<0.0001; Compounds effect: $F_{(1,24)}$=22.55, *P*<0.0001; Interaction: $F_{(5,24)}$=4.734, *P*=0.0038. VMAT2: dose-response effect: $F_{(5,24)}$=9.977, *P*<0.0001; Compounds effect: $F_{(1,24)}$=15.33, *P*=0.0007; Interaction: $F_{(5,24)}$=3.383, *P*=0.0187. TH: dose-response effect: $F_{(5,23)}$=25.09, *P*<0.0001; Compounds effect: $F_{(1,23)}$=42.11, *P*<0.0001; Interaction: $F_{(5,23)}$=12.67, *P*<0.0001**.** SYNGR3: dose-response effect: $F_{(5,23)}$=13.53, *P*=0.0012; Compounds effect: $F_{(1,23)}$=15.53, *P*=0.0167; Interaction: $F_{(5,23)}$=3.653, *P*<0.0140) followed by Bonferroni post hoc test: *P<0.05, **P<0.01, **P<0.001, ****P<0.0001, vs corresponding vehicle treated cells. B represents the basal level: cells without treatment. B, basal level.

Overall, these data suggest that the increase in VMAT2, SYNGR3, and SV2A levels are consequent to an increased transcriptional activity of SREBP. Furthermore, the observed increase for TH and SV2C proteins is independent of such transcriptional event, possibly dependent of differential protein stabilities. Finally, as modest transcription still occurs even in presence of PF-429242, our data suggest that either low level of active SREBP is enough to sustain the transcription of synaptic proteins or other factors are responsible for such baseline transcription.

### V. 4.3. SREBP-1 transcription factor is necessary to sustain the expression of VMAT2 and SV2C

Pharmacological enhancement of SREBP-1 activity provokes the differentiation towards the dopaminergic phenotype of SH-SY5Y cells (Schmitt et al., 2015). Lowering SREBP-1 levels was the next step to ascertain its direct role in such phenomenon. However, Preliminary experiments using SH-SY5Y cells did not lead to a suitable transfection level of siRNA. We thus selected the BE(2)-M17 neuroblastoma cells also known to present a pronounced dopaminergic phenotype. RT-PCR analysis confirmed the total downregulation of SREBP-1 reaching 88.9±4.1% after 72 h of incubation with the specific SiRNA while no changes in SREBP-1 levels was observed with mock SiRNA (Figure 4). The selectivity of SREBP-1 SiRNA concentrated at 25 nM was confirmed because of the expression of SREBP-2 isoform remained stable after siRNA transfection (Figure 4). SREBP-1 SiRNA treatment reduced the expression level of 70.7±3.2% and 35.1±12.1% for VMAT2 and SV2C, respectively (Figure 4). However, an increase of 176.3±25% was detected for SV2A mRNA in the downregulation of SREBP-1 (Figure 4). These findings support the involvement of SREBP-1 in the maintenance of the basal expression level of presynaptic dopaminergic protein VMAT2 and SV2C.

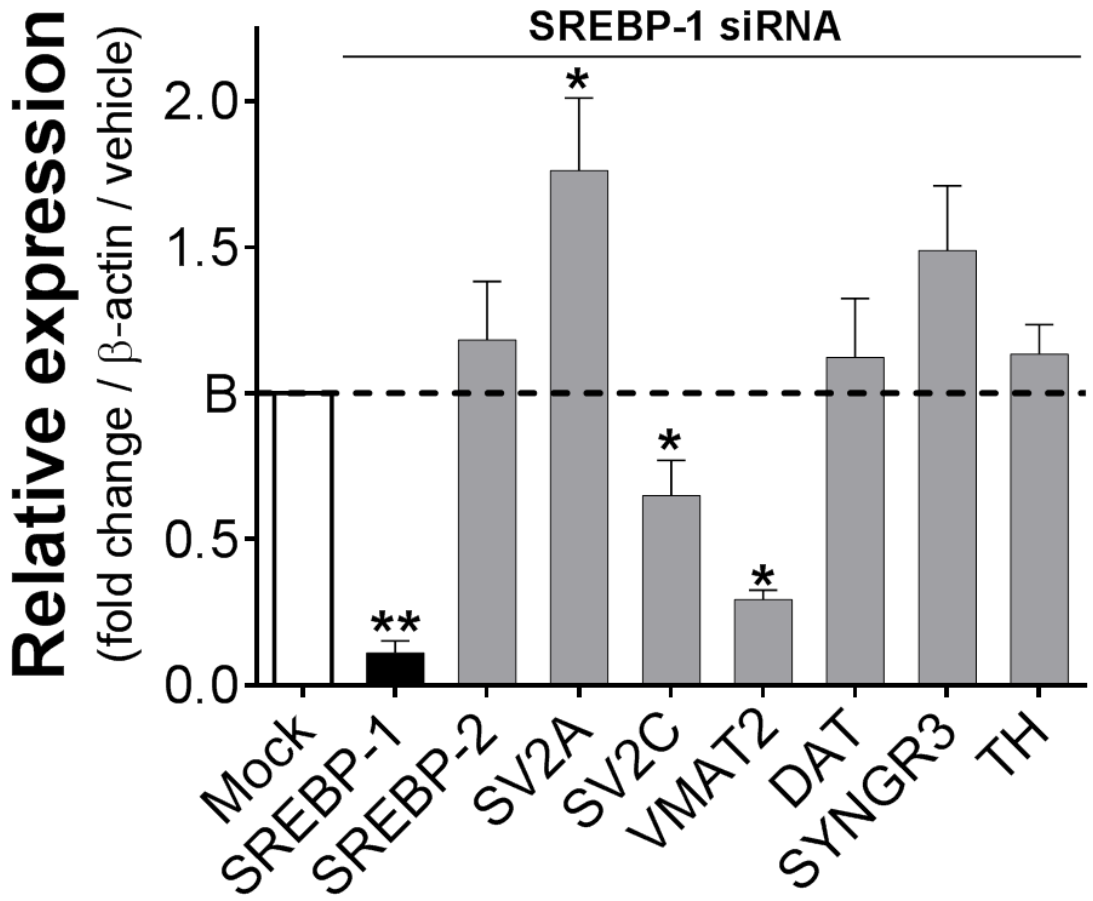


Figure 26. (Figure 4.) **Downregulation of SREBP-1 modulates the mRNA expression of presynaptic proteins VMAT2, SV2C and SV2A**

RT-PCR analysis of the dopaminergic presynaptic proteins in BE(2)-M17 treated with 25nM SREBP-1 or mock sIRNA incubated 72 h. Results represent means ± S.E.M. (n=4). Significant differences were measured by one-way ANOVA ($F_{8,27}$)=3.27, $P$<0.0098) followed by Bonferroni post hoc test: *$P$<0.05 and **$P$<0.01, vs mock SiRNA. B, basal level in mock SiRNA.

# V. 5. Discussion

Present study demonstrates that SREBP-1 nuclear translocation is associated to an increase of transcriptional expression of presynaptic markers SV2A, VMAT2 and SYNGR3 while its downregulation reduces the expression of vesicular transporter VMAT2 and SV2C.

We show here that the treatment of SH-SY5Y neuroblastoma cells with U18666A, a SREBP-1 activator, triggered SREBP-1 nuclear translocation. It has been shown that U18666A induces a reduction of cholesterol concentration and the maturation of SREBP-1 that in turn translocates to the nucleus (Lange et al., 1999; Worgall et al., 2002; Zhang et al., 2004; Colgan et al., 2007). Our data show for the first time the activation of this cholesterol pathway-dependent transcriptional mechanism. This finding agrees and extend previous report in non-neuronal cells (e.g. HELA cells) (Colgan et al., 2007). In our study, U18666A also induced the expression of SREBP demonstrating a simultaneous modulation of the transcriptional signal and of the modulator itself. The lack of effect of PF-429242, a S1P inhibitor, on SREBP-1 translocation and the reduction of its expression confirms the presence in SH-SY5Y cells of the SREBP autoregulatory mechanism also observed in other cell lines (Sato et al., 1996; Amemiya-Kudo et al., 2000; Xiao and Song, 2013). Actually, it has been shown that pharmacological activation of SREBP-1 induces its mRNA transcription through the direct binding of SREBP protein on its promoter (Sato et al., 1996; Amemiya-Kudo et al., 2000), while SREBP inactivation by PF-429242 treatment (Hawkins et al., 2008) or by knocking down S1P gene in mice causes a lower expression of SREBP (Yang et al., 2001). The slightly higher ratio of the nuclear SREBP-1 induced by PF-429242 could be explained by a reduction of immature SREBP levels into the cytoplasm.

The effects of U18666A treatment reported here are similar to our previously reported data on statin-induced SREBP-1 nuclear translocation (Schmitt et al., 2015). It is worth noting that in identical conditions U18666A efficacy is higher than that of statins (Schmitt et al., 2015). Several putative mechanism of SREBP activation could explain this difference of efficacy. Indeed, statins only inhibit the cholesterol synthesis from HMG-CoA without affecting neither its metabolism nor its transport (Sirtori, 2014) while U18666A is able to inhibit (i) the 2,3-oxidosqualene-lanosterol cyclase enzyme which is the direct upstream enzyme of the cholesterol synthesis (Mark et al., 1996; Zhang et al., 2004; Cenedella, 2009); (ii) the low density lipoprotein (LDL)-stimulated cholesterol esterification, which impairs the

transformation of the cholesteryl ester into the free cholesterol in the endoplasmic reticulum (ER) (Liscum et al., 1989; Sparrow et al., 1999); (iii) the late endosomal/lysosomal trafficking of LDL free cholesterol to the ER and Golgi (Härmälä et al., 1994; Sparrow et al., 1999; Worgall et al., 2002; Zhang et al., 2004; Cenedella, 2009). Therefore compared to statins, U18666A may completely deplete cells of free cholesterol in the ER where SREBP is located leadings to its swift and efficient translocation into the nucleus (Schmitt et al., 2015).

The present results also first demonstrate the U18666A-induced modulation of the dopaminergic synaptic markers expression. These new findings are in line with our previous study demonstrating a similar effect of statins (Schmitt et al., 2015) and agree with the reported upregulation of SV2A observed after SREBP-1 transfection in non-neuronal cells (Kallin et al., 2007). Both statins and SREBP-1 activator U18666A induce: (i) an overall up-regulation of presynaptic dopaminergic protein levels (SV2A, SV2C, VMAT2, TH and SYNGR3) and (ii) a stable increased VMAT2, SYNGR3 and SV2A mRNA expression. However, our results showing a U18666A-mediated time-dependent upregulation of VMAT2 expression suggest a delayed time frame compared to the statin-induced signalling taking place at an earlier stage (Schmitt et al., 2015). These observations suggest that the upregulation of SREBP expression induced by U18666A participates to the expression of the dopaminergic markers by modulating the gene expression levels such as VMAT2, SYNGR3 and SV2C. Our data using SREBP-1 siRNA demonstrate that the role and sensitivity of this control mechanism in the presynaptic dopaminergic phenotype. Downregulation of SREBP-1 induced a significant reduction of VMAT2 and SV2C. These new findings are in agreement with the observed regulation of these dopaminergic biomarkers induced by U18666A. Interestingly, the treatment with SREBP-1 siRNA increased the levels of SV2A. It remains unclear whether upregulation of SV2A mRNA levels induced by the SREBP-1 downregulation found here results from the selective action of SREBP-1 activity or it results from a SV2 compensation isoform mechanism triggered by the decrease of SV2C expression level (Xu and Bajjalieh, 2001).

SREBP transcriptional activity inducing an increase of VMAT2 expression could have a neuroprotective impact in PD neurodegeneration (Speciale et al., 1998; Bernstein et al., 2014; Lohr and Miller, 2014; Yulug et al., 2015). It has been shown that upregulation of VMAT2 expression reduces damages and oxidative stress through the sequestration into the vesicles of neurotoxic agents (e.g. MPTP) and cytosolic oxidative

dopamine (Speciale et al., 1998; Chen et al., 2005; Brighina et al., 2013; Lohr et al., 2014). In contrast, VMAT2 knockout mice and treatment with VMAT2 inhibitors results in an increased MPTP toxicity (Takahashi et al., 1997; Gainetdinov et al., 1998; Staal and Sonsalla, 2000). Moreover, the transcriptional control of SV2C and SYNGR3 by SREBP-1 suggested by our findings may also participate in the modulation of cytosolic dopamine levels as suggested by previous findings (Egana et al., 2009; Dardou et al., 2011, 2013; Segura-Aguilar et al., 2014).

Altogether, our results with U18666A activator are closely similar of those effects observed with the statins, which suggests that both substances use the same downstream cholesterol-dependent SREBP-1 pathway for regulation of synaptic markers (Schmitt et al., 2015). This likely excludes the involvement of downstream pathways of the statins such as PI3K/Akt and RhoA pathways in the up-regulation of dopaminergic markers (Schulz et al., 2004; Evangelopoulos et al., 2009; Racchetti et al., 2010; Jin et al., 2012; Raina et al., 2013).

In conclusion, we demonstrated the role of SREBP-1 in the expression and transcriptional activity of dopaminergic biomarkers. Altogether, our data strengthen previous findings on the regulation of dopaminergic phenotype by statins in SH-SY5Y cells. Further investigation on the cellular and molecular mechanisms driven by SREBP transcriptional activity may help to better understand its role in the regulation of dopamine phenotype as well as its potential neuroprotective and neurorestorative role of statins in Parkinson's disease. Overall, our results open new ways for neuroprotection as therapeutic uses in PD of SREBP modulators.

# V. 6. References

Amemiya-Kudo M, Shimano H, Yoshikawa T, Yahagi N, Hasty AH, Okazaki H, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Sato R, Kimura S, Ishibashi S, Yamada N (2000) Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J Biol Chem 275:31078–31085.

Brighina L, Riva C, Bertola F, Saracchi E, Fermi S, Goldwurm S, Ferrarese C (2013) Analysis of vesicular monoamine transporter 2 polymorphisms in Parkinson's disease. Neurobiol Aging 34:1712 e9–e13.

Castro AA, Wiemes BP, Matheus FC, Lapa FR, Viola GG, Santos AR, Tasca CI, Prediger RD (2013) Atorvastatin improves cognitive, emotional and motor impairments induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats, an experimental model of Parkinson's disease. Brain Res 1513:103–116.

Cenedella RJ (2009) Cholesterol synthesis inhibitor U18666A and the role of sterol metabolism and trafficking in numerous pathophysiological processes. Lipids 44:477–487.

Chen CX, Huang SY, Zhang L, Liu YJ (2005) Synaptophysin enhances the neuroprotection of VMAT2 in MPP+-induced toxicity in MN9D cells. Neurobiol Dis 19:419–426.

Colgan SM, Tang D, Werstuck GH, Austin RC (2007) Endoplasmic reticulum stress causes the activation of sterol regulatory element binding protein-2. Int J Biochem Cell Biol 39:1843–1851.

Dardou D, Dassesse D, Cuvelier L, Deprez T, De Ryck M, Schiffmann SN (2011) Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res 1367:130–145.

Dardou D, Monlezun S, Foerch P, Courade JP, Cuvelier L, De Ryck M, Schiffmann SN (2013) A role for Sv2c in basal ganglia functions. Brain Res 1507:61–73.

Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F (2004) SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86:839–848.

Egana LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Pena K, Quiroz M, Hong WC, Dorostkar MM, Janz R, Sitte HH, Torres GE (2009) Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. J Neurosci 29:4592–4604.

Engelking LJ, Liang G, Hammer RE, Takaishi K, Kuriyama H, Evers BM, Li W-P, Horton JD, Goldstein JL, Brown MS (2005) Schoenheimer effect explained--feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. J Clin Invest 115:2489–2498.

Espenshade PJ (2006) SREBPs: sterol-regulated transcription factors. J Cell Sci 119:973–976.

Evangelopoulos ME, Weis J, Kruttgen A (2009) Mevastatin-induced neurite outgrowth of neuroblastoma cells via activation of EGFR. J Neurosci Res 87:2138–2144.

Gainetdinov RR, Fumagalli F, Wang Y-M, Jones SR, Levey AI, Miller GW, Caron MG (1998) Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. J Neurochem 70:1973–1978.

Gao X, Simon KC, Schwarzschild MA, Ascherio A (2012) Prospective study of statin use and risk of Parkinson disease. Arch Neurol 69:380–384.

Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, Pahan K (2009) Simvastatin inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson's disease. J Neurosci 29:13543–13556.

Härmälä AS, Pörn MI, Mattjus P, Slotte JP (1994) Cholesterol transport from plasma membranes to intracellular membranes is inhibited by 3 beta-[2-(diethylamino)ethoxy]androst-5-en-17-one. Biochim Biophys Acta 1211:317–325.

Hawkins JL, Robbins MD, Warren LC, Xia D, Petras SF, Valentine JJ, Varghese AH, Wang I-K, Subashi TA, Shelly LD, Hay BA, Landschulz KT, Geoghegan KF, Harwood HJ (2008) Pharmacologic inhibition of site 1 protease activity inhibits sterol regulatory element-binding protein processing and reduces lipogenic enzyme gene expression and lipid synthesis in cultured cells and experimental animals. J Pharmacol Exp Ther 326:801–808.

Hay BA, Abrams B, Zumbrunn AY, Valentine JJ, Warren LC, Petras SF, Shelly LD, Xia A, Varghese AH, Hawkins JL, Van Camp JA, Robbins MD, Landschulz K, Harwood HJ (2007) Aminopyrrolidineamide inhibitors of site-1 protease. Bioorg Med Chem Lett 17:4411–4414.

Jeon T-I, Osborne TF (2012) SREBPs: metabolic integrators in physiology and metabolism. Trends Endocrinol Metab TEM 23:65–72.

Jin Y, Sui HJ, Dong Y, Ding Q, Qu WH, Yu SX, Jin YX (2012) Atorvastatin enhances neurite outgrowth in cortical neurons in vitro via up-regulating the Akt/mTOR and Akt/GSK-3beta signaling pathways. Acta Pharmacol Sin 33:861–872.

Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferre P, Heldin CH, Delzenne NM, Demoulin JB (2007) SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 gamma. J Lipid Res 48:1628–1636.

Kim JB, Spiegelman BM (1996) ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev 10:1096–1107.

Kumar A, Sharma N, Gupta A, Kalonia H, Mishra J (2012) Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. Brain Res 1471:13–22.

Lange Y, Ye J, Rigney M, Steck TL (1999) Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. J Lipid Res 40:2264–2270.

Lee YC, Lin CH, Wu RM, Lin MS, Lin JW, Chang CH, Lai MS (2013) Discontinuation of statin therapy associates with Parkinson disease: a population-based study. Neurology 81:410–416.

Liscum L, Ruggiero RM, Faust JR (1989) The intracellular transport of low density lipoprotein-derived cholesterol is defective in Niemann-Pick type C fibroblasts. J Cell Biol 108:1625–1636.

Lohr KM, Bernstein AI, Stout KA, Dunn AR, Lazo CR, Alter SP, Wang M, Li Y, Fan X, Hess EJ, Yi H, Vecchio LM, Goldstein DS, Guillot TS, Salahpour A, Miller GW (2014) Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. Proc Natl Acad Sci U A 111:9977–9982.

Mark M, Muller P, Maier R, Eisele B (1996) Effects of a novel 2,3-oxidosqualene cyclase inhibitor on the regulation of cholesterol biosynthesis in HepG2 cells. J Lipid Res 37:148–158.

Olmstead AD, Knecht W, Lazarov I, Dixit SB, Jean F (2012) Human subtilase SKI-1/S1P is a master regulator of the HCV Lifecycle and a potential host cell target for developing indirect-acting antiviral agents. PLoS Pathog 8:e1002468.

Raina V, Gupta S, Yadav S, Surolia A (2013) Simvastatin induced neurite outgrowth unveils role of cell surface cholesterol and acetyl CoA carboxylase in SH-SY5Y cells. PLoS One 8:e74547.

Roy A, Pahan K (2011) Prospects of statins in Parkinson disease. Neuroscientist 17:244–255.

Samuel F, Reddy J, Kaimal R, Segovia V, Mo H, Hynds DL (2014) Inhibiting geranylgeranylation increases neurite branching and differentially activates cofilin in cell bodies and growth cones. Mol Neurobiol 50:49–59.

Sato R, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M (1996) Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. J Biol Chem 271:26461–26464.

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108.

Schmitt M, Dehay B, Bezard E, Garcia-Ladona FJ (2015) Harnessing the Trophic and Modulatory Potential of Statins in a Dopaminergic Cell Line. Submitt J Biol Chem.

Schulz JG, Bosel J, Stoeckel M, Megow D, Dirnagl U, Endres M (2004) HMG-CoA reductase inhibition causes neurite loss by interfering with geranylgeranylpyrophosphate synthesis. J Neurochem 89:24–32.

Segura-Aguilar J, Paris I, Munoz P, Ferrari E, Zecca L, Zucca FA (2014) Protective and toxic roles of dopamine in Parkinson's disease. J Neurochem 129:898–915.

Shao W, Espenshade PJ (2012) Expanding roles for SREBP in metabolism. Cell Metab 16:414–419.

Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N (1999) Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 274:35832–35839.

Sirtori CR (2014) The pharmacology of statins. Pharmacol Res Off J Ital Pharmacol Soc 88:3–11.

Sparrow SM, Carter JM, Ridgway ND, Cook HW, Byers DM (1999) U18666A inhibits intracellular cholesterol transport and neurotransmitter release in human neuroblastoma cells. Neurochem Res 24:69–77.

Speciale SG, Liang CL, Sonsalla PK, Edwards RH, German DC (1998) The neurotoxin 1-methyl-4-phenylpyridinium is sequestered within neurons that contain the vesicular monoamine transporter. Neuroscience 84:1177–1185.

Staal RG, Sonsalla PK (2000) Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. J Pharmacol Exp Ther 293:336–342.

Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR (1997) VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci 94:9938–9943.

Wahner AD, Bronstein JM, Bordelon YM, Ritz B (2008) Statin use and the risk of Parkinson disease. Neurology 70:1418–1422.

Wolozin B, Wang SW, Li NC, Lee A, Lee TA, Kazis LE (2007) Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. BMC Med 5:20.

Worgall TS, Johnson RA, Seo T, Gierens H, Deckelbaum RJ (2002) Unsaturated fatty acid-mediated decreases in sterol regulatory element-mediated gene transcription are linked to cellular sphingolipid metabolism. J Biol Chem 277:3878–3885.

Xiao X, Song B-L (2013) SREBP: a novel therapeutic target. Acta Biochim Biophys Sin 45:2–10.

Xu T, Bajjalieh SM (2001) SV2 modulates the size of the readily releasable pool of secretory vesicles. Nat Cell Biol 3:691–698.

Xu YQ, Long L, Yan JQ, Wei L, Pan MQ, Gao HM, Zhou P, Liu M, Zhu CS, Tang BS, Wang Q (2013) Simvastatin induces neuroprotection in 6-OHDA-lesioned PC12 via the PI3K/AKT/caspase 3 pathway and anti-inflammatory responses. CNS Neurosci Ther 19:170–177.

Yabe D, Brown MS, Goldstein JL (2002) Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. Proc Natl Acad Sci U S A 99:12753–12758.

Yang J, Goldstein JL, Hammer RE, Moon YA, Brown MS, Horton JD (2001) Decreased lipid synthesis in livers of mice with disrupted Site-1 protease gene. Proc Natl Acad Sci U S A 98:13607–13612.

Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS (2002) Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell 110:489–500.

Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, Xiong Z, Deng C, Huang XF, Yenari MA, Yang YG, Ying W, Wang Q (2011) Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. PLoS One 6:e20945.

Zhang J, Dudley-Rucker N, Crowley JR, Lopez-Perez E, Issandou M, Schaffer JE, Ory DS (2004) The steroidal analog GW707 activates the SREBP pathway through disruption of intracellular cholesterol trafficking. J Lipid Res 45:223–231.

# V. 7. Supplemental data

Table 13. (Supplemental Table 1) **Pharmacological parameters of U18666A and PF-429242 dose-responses on SREBP-1 nuclear translocation in SH-SY5Y cells**

| Nuclear translocation of SREBP-1: pharmacologic parameters | | | | | | |
|---|---|---|---|---|---|---|
| **Conditions** | **U18666A** | | | **PF-429242** | | |
| **Time incubation** | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (% over vehicle) | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (% over vehicle) |
| **6 h** | Not significant over vehicle | | | Not significant over vehicle | | |
| **12 h** | 3.5 ± 2.3 | 0.6 ± 0.2 | 112.1 ± 0.4 | | | |
| **24 h** | 5.6 ± 1.9 | 0.9 ± 0.2 | 115.2 ± 2.2 | | | |
| **48 h** | 7.0 ± 0.5 | 2.0 ± 0.2 | 158.3 ± 3.4 | 8.7 ± 1.52 | 1.59 ± 0.37 | 117.4 ± 2.3 |

SREBP-1 compound dose-responses up to 30µM were incubated in SH-SY5Y cells.
Results represent the means ± S.E.M. (n=3) and pharmacologic parameters were determined by nonlinear regression with a 4PL and normalized fitting of fluorescence intensity ratio between cytoplasm and nucleus in SREBP-1 immunocytochemistry (background subtracted). Significant differences were measured by two-way ANOVA (U18666A, treatment $F_{(5,24)}$=192.6, *P*<0.0001; Time: $F_{(3,24)}$=239.8, *P*<0.0001; Interaction: $F_{(15,24)}$=76.43, *P*<0.0001; PF-429242 treatment: $F_{(5,48)}$=10.06, *P*<0.0001; Time: $F_{(3,48)}$=13.56, *P*<0.0001; Interaction: $F_{(15,48)}$=2.742, *P*<0.0001).

Table 14. (Supplemental Table 2) **Pharmacological parameters of U18666A and PF-429242 dose-responses on the dopaminergic markers expression level in SH-SY5Y cells**

| Presynaptic dopaminergic protein expression level: pharmacologic parameters | | | | | | |
|---|---|---|---|---|---|---|
| **Conditions** | **U18666A do** | | | **PF-429242** | | |
| **Proteins** | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (% over vehicle) | **$EC_{50}$** (µM) | **$n_h$** | **$E_{max}$** (% over vehicle) |
| **SV2A** | 12.2 ± 4.2 | 2.7 ± 2.3 | 326.3 ± 55.3 | Not significant over vehicle | | |
| **SV2C** | 8.3 ± 3.4 | 1.6 ± 0.8 | 363.4 ± 57.3 | | | |
| **VMAT2** | 10.2 ± 3 | 1.6 ± 0.6 | 324.0 ± 66.5 | | | |
| **TH** | 11.5 ± 3.1 | 2.0 ± 0.9 | 321.2 ± 41.9 | | | |
| **SYNGR3** | 6.6 ± 1.8 | 2.0 ± 0.8 | 246.0 ± 69.4 | | | |

SREBP-1 compound dose-responses up to 30µM were incubated 48 h in SH-SY5Y cells.
Results represent the means ± S.E.M. (n=3) and pharmacologic parameters were determined by nonlinear regression of fluorescence intensity in immunocytochemistry assay reported in Figure 3. Significant differences were measured by two-way ANOVA (SV2A, dose-response effect: $F_{(5,24)}$=16.77, *P*<0.0001; Compound effect: $F_{(1,24)}$=20.06, *P*=0.0002; Interaction: $F_{(5,24)}$=6.274, *P*=0.0007. SV2C, dose-response effect: $F_{(5,24)}$=18.97, *P*<0.0001; Compound effect: $F_{(1,24)}$=22.55, *P*<0.0001; Interaction: $F_{(5,24)}$=4.734, *P*=0.0038. VMAT2: dose-response effect: $F_{(5,24)}$=9.977, *P*<0.0001; Compound effect: $F_{(1,24)}$=15.33, *P*=0.0007; Interaction: $F_{(5,24)}$=3.383, *P*=0.0187. TH: dose-response effect: $F_{(5,23)}$=25.09, *P*<0.0001; Compound effect: $F_{(1,23)}$=42.11, *P*<0.0001; Interaction: $F_{(5,23)}$=12.67, *P*<0.0001. SYNGR3: dose-response effect: $F_{(5,23)}$=13.53, *P*=0.0012; Compounds effect: $F_{(1,23)}$=15.53, *P*=0.0167; Interaction: $F_{(5,23)}$=3.653, *P*<0.0140).

# Conclusions and perspectives

For several decades, the drug treatment of PD aiming to replace the massive pathological deficit of dopamine has been a major therapeutic success because it alleviates the motor symptoms and represents a huge benefit for patients (Giugni and Okun, 2014). However, today there is no cure for the progression of neurodegenerative process. Several therapeutic strategies are currently under consideration such as neuroprotection and neurorestoration, aiming to halt disease progression and to recover locomotor functions and normal physiological conditions (Henchcliffe and Severt, 2011). All these so-called disease-modifying treatments are in the preclinical stages or early in clinical trials. Unfortunately, the first approaches aiming to stop the neurodegenerative processes (rasagiline) have not been fully satisfactory in clinical studies and their neuroprotective potential remains to be fully demonstrated (Henchcliffe and Severt, 2011). In this context, there is an unmet clinical need priming for searching new therapeutic targets and treatment strategies as well as for identifying new preclinical models for PD with a stronger translational potential (Martinez, 2014). Moreover, a better understanding of the neurodegenerative mechanism underlying the disease and its progression remains a major challenge for targeting better therapeutic approaches (Brundin et al., 2015).

During the last decade, an intense effort of discovery research in PD intends to identify therapeutic approaches for modifying the course of the disease (Brundin et al., 2015). Several signalling pathways have been shown as important intervention points for stopping or slowing down neurodegeneration in PD (Ramanan and Saykin, 2013). The targets that modulate either the neurotrophic or neuroprotective mechanism or both could be of major interest because of their potentials for protecting the dopaminergic cells against neurotoxic challenges and for inducing the neurorestoration of lost cells and synaptic connexions (Blaudin de Thé et al., 2015). Among others, neurotrophic factors (e.g. GDNF, BDNF) and transcription factors modulating neurodifferentiation (e.g. NURR1 and RAR) are an area of major interest in PD research (Blaudin de Thé et al., 2015). In this context, the cholesterol pathway and more particularly the inhibition of HMG-CoA by statins has been proposed to have a significant therapeutic potential for neuroprotection and neurorestoration in neurological diseases, especially in PD, and has been the main focus of this project (Reiss and Wirkowski, 2007).

Epidemiological studies and preclinical researches have showed the neuroprotective potential of statins, which suggests that the medical use of statins may reduce the risk of developing PD (Becker and Meier, 2009). Compelling data demonstrate that statins have neuroprotective effects by reducing dopaminergic cell death in cultured systems as well as in animals (van der Most et al., 2009). The suggested pathways and mechanisms leading to statin-induced neuroprotective include a reduction of oxidative stress, neuroinflammation and aggregation of α-synuclein (Roy and Pahan, 2011); all of them are processes demonstrated to be increased in PD pathology (Yacoubian and Standaert, 2009). The potential neurorestorative effect of statins remains poorly investigated. Thus, we aimed to determine the effect of statins and the associated downstream signalling pathways in the genesis of synaptic structures and in the expression and functionality of presynaptic dopaminergic markers. The data generated in the present study demonstrate new regulatory effects of statins in the neuritogenesis, synaptic protein expression and function of dopaminergic transport system in the human dopaminergic neuroblastoma SH-SY5Y cell line.

The neurotrophic effects of statins found in our study confirm and extend previous data showing statin-induced neurite growth (Raina et al., 2013). We demonstrate that statins induce neurite branching rather primary neurite elongation, an effect that differentiates these compounds from other neurotrophic principles. Statin-reported effects on neurite growth could be mediated by downstream impact in the RhoA system or by indirect interference with PI3K/Akt pathway. Both have been shown to affect branching complexity of neuronal processes (Higuchi et al., 2003; Hynds et al., 2003; Petrinovic et al., 2010). Combination experiments demonstrated that the effects of statin in our study are, at least in part, independent of RhoA system and fully independent of PI3K pathways. Nevertheless, we have demonstrated that statin treatment induced a reversal of neurite retraction induced by inhibitors of the PI3K pathway. However, we cannot elucidate the role of the enhancement of synaptic protein expression or the decrease of cholesterol by the statins on the neurite growth and branching complexity (Tarsa and Goda, 2002; Pfrieger, 2003; Wang et al., 2008). Overall, the effects of statins on neurite growth demonstrate a neurorestorative and neuroprotective potential of these compounds against the axonal degeneration in PD.

In addition to the effects of statins in neurite growth of SH-SY5Y cells, we have found a significant increase in the levels of presynaptic markers of the dopaminergic system. Synaptic vesicle proteins levels such as SV2C, SV2A, VMAT2 and SYNGR3 were increased

after statin treatment. We could demonstrate that the increases of protein level were associated to gene expression up-regulation for the vesicular transporter SV2C and VMAT2. Further investigation allowed us to strengthen the direct implication of cholesterol downstream pathway in these phenotypic changes by demonstrating the effects of SREBP direct modulators in protein levels of VMAT2, SV2C, SYNGR3 and SV2A. The activation of SREBP genes by the nuclear factor LXR using specific ligand could be an interesting way to measure the involvement of SREBP in the regulation of synaptic proteins (Repa et al., 2000; Yoshikawa et al., 2001). Furthermore, our findings obtained by using siRNA suggest that SREBP-1 may be necessary for the expression of SV2C and VMAT2. In summary, our data suggest that the changes in synaptic protein markers are a unique modulatory action of statins on not only the dopaminergic phenotype, but also on other neurotransmitter systems. To our knowledge, this effect has not been yet reported.

An important objective of our project was to determine the impact of statins in the function of the dopamine transport system. Our findings demonstrate statin-induced decrease of total dopamine transport capacity and specific changes in the pharmacological sensitivity of VMAT2. Our findings suggest that the decrease of dopamine uptake could be likely associated to an imbalance in the dopamine transport cycle likely affected by changes in DAT and VMAT2 pharmacology and expression levels as well as by the differential increase in the protein levels of SV2C and SYNGR3. However, these data could also be in line with previous reports showing that a low cholesterol level in lipid rafts reduce the DAT uptake capacity (Adkins et al., 2007; Cremona et al., 2011). Moreover, activation of PI3K pathway has been shown to modulate DAT internalization by phosphorylation and could lead to less DAT at the membrane surface and subsequent a reduction transport capacity (Torres et al., 2003b). In this context, we could suggest that statins can mediate the internalization of DAT by PI3K pathway activation. This remains to be confirmed by measuring the levels of DAT at cell surface and its phosphorylation levels.

In summary, the present project aimed to study the effect of statins in the biology of dopaminergic system. The findings obtained in present project demonstrate the modulation by statins of A) the genesis of neuronal synaptic elements B) the expression and/or the levels of dopaminergic system proteins VMAT2, SYNGR3, SV2C, DAT, TH and C) the modulation of functional dopamine transport cycle. Investigation of downstream pathways involved in above mechanisms allowed us to also show that neurotrophic effects of statins are

partially independent of RhoA and PI3K pathways while a SREBP-dependent pathway is involved in the modulation of dopaminergic system biomarkers. Overall, these results suggest a therapeutic action of statins against neurodegenerative processes taking place in PD. The neuroprotective and neurotrophic effects of statins as well as their impact in the function of dopamine transport extend the previous studies which have shown efficacy in oxidative stress and neuroinflammation and strengthen the suggested therapeutic potential of statins in PD.

The results obtained during the development of the present project have contributed to identify new mechanisms and downstream pathways triggered by the treatment of SH-SY5Y neuroblastoma cells by statins. However, the findings have open new questions that should be investigated to understand the biology of cholesterol pathway and its impact on neurodegenerative processes and on the therapeutic potential of statins. Further investigations could be triggered regarding the neurorestorative effects of the statins. It would be important to know whether statin have neurorestorative effect by the unique impact in the network complexity or by the regulation of the synapse's functionality. In this context, the investigation of the molecular impacts mediated by statins on dopaminergic synaptic proteins, such as protein turnover, membrane residence and post-transcriptional modifications may help to explain the modulation of the dopamine transport cycle. The importance of cholesterol pathway for the integrity and multiple functionalities of protein anchored in the neuronal cell membranes support additional research in order to demonstrate the regulatory points possibly requires at transcriptional levels in the SREBP pathway. Moreover, it would be very important to demonstrate that such effects could be observed in PD animal models and particularly if they could hinder and reverse neurodegenerative mechanisms, and if so, they may add translational value for the human neurodegeneration.

# References

Adinoff B (2004) Neurobiologic processes in drug reward and addiction. Harv Rev Psychiatry 12:305–320.

Adkins EM, Samuvel DJ, Fog JU, Eriksen J, Jayanthi LD, Vaegter CB, Ramamoorthy S, Gether U (2007) Membrane mobility and microdomain association of the dopamine transporter studied with fluorescence correlation spectroscopy and fluorescence recovery after photobleaching. Biochemistry (Mosc) 46:10484–10497.

Afonso-Oramas D, Cruz-Muros I, Alvarez de la Rosa D, Abreu P, Giráldez T, Castro-Hernández J, Salas-Hernández J, Lanciego JL, Rodríguez M, González-Hernández T (2009) Dopamine transporter glycosylation correlates with the vulnerability of midbrain dopaminergic cells in Parkinson's disease. Neurobiol Dis 36:494–508.

Ahnert-Hilger G, Nürnberg B, Exner T, Schäfer T, Jahn R (1998) The heterotrimeric G protein Go2 regulates catecholamine uptake by secretory vesicles. EMBO J 17:406–413.

Airavaara M, Voutilainen MH, Wang Y, Hoffer B (2012) Neurorestoration. Parkinsonism Relat Disord 18 Suppl 1:S143–S146.

Alabi AA, Tsien RW (2012) Synaptic vesicle pools and dynamics. Cold Spring Harb Perspect Biol 4:a013680.

Alberts AW et al. (1980) Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci U S A 77:3957–3961.

Alberts AW (1988) Discovery, biochemistry and biology of lovastatin. Am J Cardiol 62:10J – 15J.

Alberts AW (1990) Lovastatin and simvastatin--inhibitors of HMG CoA reductase and cholesterol biosynthesis. Cardiology 77 Suppl 4:14–21.

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.

Alcaro A, Huber R, Panksepp J (2007) Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective. Brain Res Rev 56:283–321.

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.

Allodi I, Hedlund E (2014) Directed midbrain and spinal cord neurogenesis from pluripotent stem cells to model development and disease in a dish. Front Neurosci 8:109.

Alter SP, Lenzi GM, Bernstein AI, Miller GW (2013) Vesicular Integrity in Parkinson's Disease. Curr Neurol Neurosci Rep 13 Available at: http://link.springer.com/10.1007/s11910-013-0362-3 [Accessed July 16, 2015].

Amemiya-Kudo M, Shimano H, Yoshikawa T, Yahagi N, Hasty AH, Okazaki H, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Sato R, Kimura S, Ishibashi S, Yamada N (2000) Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J Biol Chem 275:31078–31085.

Anderson KE (2004) Behavioral disturbances in Parkinson's disease. Dialogues Clin Neurosci 6:323–332.

Andersson S, Gustafsson N, Warner M, Gustafsson J-A (2005) Inactivation of liver X receptor beta leads to adult-onset motor neuron degeneration in male mice. Proc Natl Acad Sci U S A 102:3857–3862.

Arenas E (2008) Foxa2: the rise and fall of dopamine neurons. Cell Stem Cell 2:110–112.

Ariano MA (1983) Distribution of components of the guanosine 3',5'-phosphate system in rat caudate-putamen. Neuroscience 10:707–723.

Arimura N, Menager C, Fukata Y, Kaibuchi K (2004) Role of CRMP-2 in neuronal polarity. J Neurobiol 58:34–47.

Arrieta O, García-Navarrete R, Zúñiga S, Ordóñez G, Ortiz A, Palencia G, Morales-Espinosa D, Hernández-Pedro N, Sotelo J (2005) Retinoic acid increases tissue and plasma contents of nerve growth factor and prevents neuropathy in diabetic mice. Eur J Clin Invest 35:201–207.

Asanuma M, Miyazaki I, Diaz-Corrales FJ, Ogawa N (2004) Quinone formation as dopaminergic neuron-specific oxidative stress in the pathogenesis of sporadic Parkinson's disease and neurotoxin-induced parkinsonism. Acta Med Okayama 58:221–233.

Bae E-J, Lee H-S, Park C-H, Lee S-H (2009) Orphan nuclear receptor Nurr1 induces neuron differentiation from embryonic cortical precursor cells via an extrinsic paracrine mechanism. FEBS Lett 583:1505–1510.

Bajjalieh SM, Frantz GD, Weimann JM, McConnell SK, Scheller RH (1994) Differential expression of synaptic vesicle protein 2 (SV2) isoforms. J Neurosci Off J Soc Neurosci 14:5223–5235.

Bajjalieh SM, Peterson K, Linial M, Scheller RH (1993) Brain contains two forms of synaptic vesicle protein 2. Proc Natl Acad Sci U S A 90:2150–2154.

Bannon MJ (2005) The dopamine transporter: role in neurotoxicity and human disease. Toxicol Appl Pharmacol 204:355–360.

Bannon MJ, Whitty CJ (1997) Age-related and regional differences in dopamine transporter mRNA expression in human midbrain. Neurology 48:969–977.

Barker RA, Barrett J, Mason SL, Björklund A (2013) Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. Lancet Neurol 12:84–91.

Bar-On P, Crews L, Koob AO, Mizuno H, Adame A, Spencer B, Masliah E (2008) Statins reduce neuronal alpha-synuclein aggregation in in vitro models of Parkinson's disease. J Neurochem 105:1656–1667.

Bayer VE, Pickel VM (1990) Ultrastructural localization of tyrosine hydroxylase in the rat ventral tegmental area: relationship between immunolabeling density and neuronal associations. J Neurosci Off J Soc Neurosci 10:2996–3013.

Beaulieu J-M, Espinoza S, Gainetdinov RR (2015) Dopamine receptors - IUPHAR Review 13. Br J Pharmacol 172:1–23.

Beaulieu J-M, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63:182–217.

Becker C, Meier CR (2009) Statins and the risk of Parkinson disease: an update on the controversy. Expert Opin Drug Saf 8:261–271.

Beilina A, Cookson MR (2015) Genes associated with Parkinson's disease: regulation of autophagy and beyond. J Neurochem.

Beitz JM (2014) Parkinson's disease: a review. Front Biosci Sch Ed 6:65–74.

Belin AC, Westerlund M (2008) Parkinson's disease: a genetic perspective. FEBS J 275:1377–1383.

Belizaire R, Komanduri C, Wooten K, Chen M, Thaller C, Janz R (2004) Characterization of synaptogyrin 3 as a new synaptic vesicle protein. J Comp Neurol 470:266–281.

Bellucci A, Zaltieri M, Navarria L, Grigoletto J, Missale C, Spano P (2012) From α-synuclein to synaptic dysfunctions: New insights into the pathophysiology of Parkinson's disease. Brain Res 1476:183–202.

Benazzouz A, Gross C, Féger J, Boraud T, Bioulac B (1993) Reversal of rigidity and improvement in motor performance by subthalamic high-frequency stimulation in MPTP-treated monkeys. Eur J Neurosci 5:382–389.

Bengoechea-Alonso MT, Ericsson J (2007) SREBP in signal transduction: cholesterol metabolism and beyond. Curr Opin Cell Biol 19:215–222.

Bennett BD, Bolam JP (1994) Synaptic input and output of parvalbumin-immunoreactive neurons in the neostriatum of the rat. Neuroscience 62:707–719.

Bensinger SJ, Tontonoz P (2009) A Nurr1 pathway for neuroprotection. Cell 137:26–28.

Berardelli A, Rothwell JC, Thompson PD, Hallett M (2001) Pathophysiology of bradykinesia in Parkinson's disease. Brain J Neurol 124:2131–2146.

Berg JM, Tymoczko JL, Stryer L, Stryer L (2002) Biochemistry, 5th ed. New York: W.H. Freeman.

Bernard O (2007) Lim kinases, regulators of actin dynamics. Int J Biochem Cell Biol 39:1071–1076.

Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (1973) Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 20:415–455.

Bernstein AI, Stout KA, Miller GW (2014) The vesicular monoamine transporter 2: an underexplored pharmacological target. Neurochem Int 73:89–97.

Bertler A, Rosengren E (1959) Occurrence and distribution of catechol amines in brain. Acta Physiol Scand 47:350–361.

Berwick DC, Harvey K (2013) LRRK2: an éminence grise of Wnt-mediated neurogenesis? Front Cell Neurosci 7:82.

Bessler H, Djaldetti R, Salman H, Bergman M, Djaldetti M (1999) IL-1 beta, IL-2, IL-6 and TNF-alpha production by peripheral blood mononuclear cells from patients with Parkinson's disease. Biomed Pharmacother Bioméd Pharmacothérapie 53:141–145.

Beyenbach KW, Wieczorek H (2006) The V-type H+ ATPase: molecular structure and function, physiological roles and regulation. J Exp Biol 209:577–589.

Bezard E, Gross CE (1998) Compensatory mechanisms in experimental and human parkinsonism: towards a dynamic approach. Prog Neurobiol 55:93–116.

Bezard E, Gross CE, Brotchie JM (2003) Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. Trends Neurosci 26:215–221.

Bigornia L, Allen CN, Jan CR, Lyon RA, Titeler M, Schneider AS (1990) D2 dopamine receptors modulate calcium channel currents and catecholamine secretion in bovine adrenal chromaffin cells. J Pharmacol Exp Ther 252:586–592.

Binda F, Dipace C, Bowton E, Robertson SD, Lute BJ, Fog JU, Zhang M, Sen N, Colbran RJ, Gnegy ME, Gether U, Javitch JA, Erreger K, Galli A (2008) Syntaxin 1A Interaction with the Dopamine Transporter Promotes Amphetamine-Induced Dopamine Efflux. Mol Pharmacol 74:1101–1108.

Bin-Umer MA, McLaughlin JE, Butterly MS, McCormick S, Tumer NE (2014) Elimination of damaged mitochondria through mitophagy reduces mitochondrial oxidative stress and increases tolerance to trichothecenes. Proc Natl Acad Sci U S A 111:11798–11803.

Birkmayer W, Hornykiewicz O (1961) [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. Wien Klin Wochenschr 73:787–788.

Birkmayer W, Hornykiewicz O (1962) Der L-Dioxyphenylalanin (=L-DOPA)-Effekt beim Parkinson-Syndrom des Menschen: Zur Pathogenese und Behandlung der Parkinson-Akinese. Arch F�r Psychiatr Nervenkrankh Ver Mit Z F�r Gesamte Neurol Psychiatr 203:560–574.

Björkhem I (2006) Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. J Intern Med 260:493–508.

Björkhem I, Lütjohann D, Diczfalusy U, Ståhle L, Ahlborg G, Wahren J (1998) Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. J Lipid Res 39:1594–1600.

Björkhem I, Meaney S (2004) Brain cholesterol: long secret life behind a barrier. Arterioscler Thromb Vasc Biol 24:806–815.

Blackstone C (2009) Infantile parkinsonism-dystonia: a dopamine "transportopathy." J Clin Invest 119:1455–1458.

Blakely RD, Edwards RH (2012) Vesicular and plasma membrane transporters for neurotransmitters. Cold Spring Harb Perspect Biol 4.

Blandini F, Nappi G, Tassorelli C, Martignoni E (2000) Functional changes of the basal ganglia circuitry in Parkinson's disease. Prog Neurobiol 62:63–88.

Blaschko (1939) PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY: 8 July 1939. J Physiol 96:33–60.

Blaudin de Thé F-X, Rekaik H, Prochiantz A, Fuchs J, Joshi RL (2015) Neuroprotective Transcription Factors in Animal Models of Parkinson Disease. Neural Plast.

Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR (2015) Oxidative stress and Parkinson's disease. Front Neuroanat 9:91.

Bloem BR (1992) Postural instability in Parkinson's disease. Clin Neurol Neurosurg 94 Suppl:S41–S45.

Boje KM, Arora PK (1992) Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain Res 587:250–256.

Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC (1994) Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett 172:151–154.

Bolam JP, Smith Y (1990) The GABA and substance P input to dopaminergic neurones in the substantia nigra of the rat. Brain Res 529:57–78.

Bonifácio MJ, Palma PN, Almeida L, Soares-da-Silva P (2007) Catechol-O-methyltransferase and its inhibitors in Parkinson's disease. CNS Drug Rev 13:352–379.

Bonnet AM, Jutras MF, Czernecki V, Corvol JC, Vidailhet M (2012) Nonmotor symptoms in Parkinson's disease in 2012: relevant clinical aspects. Park Dis 2012:198316.

Borrajo A, Rodriguez-Perez AI, Villar-Cheda B, Guerra MJ, Labandeira-Garcia JL (2014) Inhibition of the microglial response is essential for the neuroprotective effects of Rho-kinase inhibitors on MPTP-induced dopaminergic cell death. Neuropharmacology 85:1–8.

Bosco DA, Fowler DM, Zhang Q, Nieva J, Powers ET, Wentworth P, Lerner RA, Kelly JW (2006) Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. Nat Chem Biol 2:249–253.

Bougria M, Vitorica J, Cano J, Machado A (1995) Implication of dopamine transporter system on 1-methyl-4-phenylpyridinium and rotenone effect in striatal synaptosomes. Eur J Pharmacol 291:407–415.

Bower JH, Maraganore DM, McDonnell SK, Rocca WA (1999) Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976-1990. Neurology 52:1214–1220.

Boyken J, Grønborg M, Riedel D, Urlaub H, Jahn R, Chua JJE (2013) Molecular profiling of synaptic vesicle docking sites reveals novel proteins but few differences between glutamatergic and GABAergic synapses. Neuron 78:285–297.

Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U (2002) Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 249 Suppl 3:III/1–5.

Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211.

Brighina L, Riva C, Bertola F, Saracchi E, Fermi S, Goldwurm S, Ferrarese C (2013) Analysis of vesicular monoamine transporter 2 polymorphisms in Parkinson's disease. Neurobiol Aging 34:1712 e9–e13.

Brooks DJ (2000) Dopamine agonists: their role in the treatment of Parkinson's disease. J Neurol Neurosurg Psychiatry 68:685–689.

Brooks DJ (2004) Neuroimaging in Parkinson's disease. NeuroRx J Am Soc Exp Neurother 1:243–254.

Brown MS, Goldstein JL (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340.

Brundin P, Atkin G, Lamberts JT (2015) Basic science breaks through: New therapeutic advances in Parkinson's disease. Mov Disord Off J Mov Disord Soc 30:1521–1527.

Brundin P, Barker RA, Parmar M (2010) Neural grafting in Parkinson's disease Problems and possibilities. Prog Brain Res 184:265–294.

Brunet A, Datta SR, Greenberg ME (2001) Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr Opin Neurobiol 11:297–305.

Brunk I, Blex C, Rachakonda S, Höltje M, Winter S, Pahner I, Walther DJ, Ahnert-Hilger G (2006) The first luminal domain of vesicular monoamine transporters mediates G-protein-dependent regulation of transmitter uptake. J Biol Chem 281:33373–33385.

Buhaescu I, Izzedine H (2007) Mevalonate pathway: a review of clinical and therapeutical implications. Clin Biochem 40:575–584.

Burchell VS, Nelson DE, Sanchez-Martinez A, Delgado-Camprubi M, Ivatt RM, Pogson JH, Randle SJ, Wray S, Lewis PA, Houlden H, Abramov AY, Hardy J, Wood NW, Whitworth AJ, Laman H, Plun-Favreau H (2013) The Parkinson's disease-linked proteins Fbxo7 and Parkin interact to mediate mitophagy. Nat Neurosci 16:1257–1265.

Burke RE, O'Malley K (2013) Axon degeneration in Parkinson's disease. Exp Neurol 246:72–83.

Burkhard P, Dominici P, Borri-Voltattorni C, Jansonius JN, Malashkevich VN (2001) Structural insight into Parkinson's disease treatment from drug-inhibited DOPA decarboxylase. Nat Struct Biol 8:963–967.

Burré J, Volknandt W (2007) The synaptic vesicle proteome. J Neurochem 101:1448–1462.

Cabbat FS, Manzino L, Heikkila RE (1985) Studies on the stability of 3H-dopamine, 3H-apomorphine and 3H-ADTN: effects of sodium ascorbate and EDTA. Res Commun Chem Pathol Pharmacol 47:333–343.

Cadet JL, Jayanthi S, McCoy MT, Beauvais G, Cai NS (2010) Dopamine D1 receptors, regulation of gene expression in the brain, and neurodegeneration. CNS Neurol Disord Drug Targets 9:526–538.

Calderón-Garcidueñas L, Solt AC, Henríquez-Roldán C, Torres-Jardón R, Nuse B, Herritt L, Villarreal-Calderón R, Osnaya N, Stone I, García R, Brooks DM, González-Maciel A, Reynoso-Robles R, Delgado-Chávez R, Reed W (2008) Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol 36:289–310.

Campbell AW, Schlesinger E, Riley HA (1905) Histological Studies on the Localisation of Cerebral Function. Cambridge: University Press.

Campolo M, Paterniti I, Siracusa R, Bruschetta G, Cuzzocrea S, Esposito E (2014) Liver X receptors activation decreases neuroinflammation in Parkinson's disease (846.5). FASEB J.

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT (2009) A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 34:279–290.

Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296:1655–1657.

Cao Z, Gao Y, Deng K, Williams G, Doherty P, Walsh FS (2010) Receptors for myelin inhibitors: Structures and therapeutic opportunities. Mol Cell Neurosci 43:1–14.

Cappelletti G, Maggioni MG, Maci R (1999) Influence of MPP+ on the state of tubulin polymerisation in NGF-differentiated PC12 cells. J Neurosci Res 56:28–35.

Cappelletti G, Surrey T, Maci R (2005) The parkinsonism producing neurotoxin MPP+ affects microtubule dynamics by acting as a destabilising factor. FEBS Lett 579:4781–4786.

Carlezon WA, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci 28:436–445.

Carlsson A, Hillarp NA, Waldeck B (1962) A Mg-ATP dependent storage mechanism in the amine granules of the adrenal medulla. Med Exp Int J Exp Med 6:47–53.

Carlsson A, Lindqvist M, Magnusson T, Waldeck B (1958) On the presence of 3-hydroxytyramine in brain. Science 127:471.

Carracedo A, Pandolfi PP (2008) The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene 27:5527–5541.

Cartagena CM, Burns MP, Rebeck GW (2010) 24S-hydroxycholesterol effects on lipid metabolism genes are modeled in traumatic brain injury. Brain Res 1319:1–12.

Cartier EA, Parra LA, Baust TB, Quiroz M, Salazar G, Faundez V, Egaña L, Torres GE (2010) A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. J Biol Chem 285:1957–1966.

Carvelli L, Blakely RD, DeFelice LJ (2008) Dopamine transporter/syntaxin 1A interactions regulate transporter channel activity and dopaminergic synaptic transmission. Proc Natl Acad Sci U S A 105:14192–14197.

Carvelli L, Morón JA, Kahlig KM, Ferrer JV, Sen N, Lechleiter JD, Leeb-Lundberg LM, Merrill G, Lafer EM, Ballou LM, others (2002) PI 3-kinase regulation of dopamine uptake. J Neurochem 81:859–869.

Casey PJ (1992) Biochemistry of protein prenylation. J Lipid Res 33:1731–1740.

Cash R, Dennis T, L'Heureux R, Raisman R, Javoy-Agid F, Scatton B (1987) Parkinson's disease and dementia: norepinephrine and dopamine in locus ceruleus. Neurology 37:42–46.

Castillo SO, Baffi JS, Palkovits M, Goldstein DS, Kopin IJ, Witta J, Magnuson MA, Nikodem VM (1998) Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. Mol Cell Neurosci 11:36–46.

Castro AA, Wiemes BP, Matheus FC, Lapa FR, Viola GG, Santos AR, Tasca CI, Prediger RD (2013) Atorvastatin improves cognitive, emotional and motor impairments induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats, an experimental model of Parkinson's disease. Brain Res 1513:103–116.

Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, Colebrooke RE, Di Monte DA, Emson PC, Miller GW (2007) Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J Neurosci Off J Soc Neurosci 27:8138–8148.

Cenedella RJ (2009) Cholesterol synthesis inhibitor U18666A and the role of sterol metabolism and trafficking in numerous pathophysiological processes. Lipids 44:477–487.

Cerruti C, Walther DM, Kuhar MJ, Uhl GR (1993) Dopamine transporter mRNA expression is intense in rat midbrain neurons and modest outside midbrain. Brain Res Mol Brain Res 18:181–186.

Cervinski MA, Foster JD, Vaughan RA (2010) Syntaxin 1A regulates dopamine transporter activity, phosphorylation and surface expression. Neuroscience 170:408–416.

Chahine LM, Stern MB (2011) Diagnostic markers for Parkinson's disease. Curr Opin Neurol 24:309–317.

Cha JY, Repa JJ (2007) The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. J Biol Chem 282:743–751.

Chan D, Citro A, Cordy JM, Shen GC, Wolozin B (2011) Rac1 Protein Rescues Neurite Retraction Caused by G2019S Leucine-rich Repeat Kinase 2 (LRRK2). J Biol Chem 286:16140–16149.

Chang TY, Chang CC, Cheng D (1997) Acyl-coenzyme A:cholesterol acyltransferase. Annu Rev Biochem 66:613–638.

Chan T, Chow AM, Cheng XR, Tang DWF, Brown IR, Kerman K (2012) Oxidative stress effect of dopamine on α-synuclein: electroanalysis of solvent interactions. ACS Chem Neurosci 3:569–574.

Charcot J-M (1872) De la paralysie agitante. In Oeuvres Complètes (t 1) Leçons sur les maladies du système nerveux. Paris.

Chartier-Harlin M-C et al. (2011) Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet 89:398–406.

Chaudhry FA, Boulland J-L, Jenstad M, Bredahl MKL, Edwards RH (2008) Pharmacology of neurotransmitter transport into secretory vesicles. Handb Exp Pharmacol:77–106.

Chaudhuri KR, Healy DG, Schapira AHV, National Institute for Clinical Excellence (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol 5:235–245.

Chauhan A, Jeans AF (2015) Is Parkinson's disease truly a prion-like disorder? An appraisal of current evidence. Neurol Res Int 2015:345285.

Chen CX, Huang SY, Zhang L, Liu YJ (2005a) Synaptophysin enhances the neuroprotection of VMAT2 in MPP+-induced toxicity in MN9D cells. Neurobiol Dis 19:419–426.

Chen CX-Q, Huang SY, Zhang L, Liu Y-J (2005b) Synaptophysin enhances the neuroprotection of VMAT2 in MPP+-induced toxicity in MN9D cells. Neurobiol Dis 19:419–426.

Cheng H-C, Ulane CM, Burke RE (2010) Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol 67:715–725.

Chen G, Liang G, Ou J, Goldstein JL, Brown MS (2004) Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc Natl Acad Sci U A 101:11245–11250.

Chen J, Chopp M (2006) Neurorestorative treatment of stroke: cell and pharmacological approaches. NeuroRx J Am Soc Exp Neurother 3:466–473.

Chen J, Song J, Yuan P, Tian Q, Ji Y, Ren-Patterson R, Liu G, Sei Y, Weinberger DR (2011a) Orientation and cellular distribution of membrane-bound catechol-O-methyltransferase in cortical neurons: implications for drug development. J Biol Chem 286:34752–34760.

Chen J, Zhang C, Jiang H, Li Y, Zhang L, Robin A, Katakowski M, Lu M, Chopp M (2005c) Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab 25:281–290.

Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, Zhang C, Lu M, Katakowski M, Feldkamp CS, others (2003) Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann Neurol 53:743–751.

Chen K-H, Wu R-M, Lin H-I, Tai C-H, Lin C-H (2015) Mutational analysis of SYNJ1 gene (PARK20) in Parkinson's disease in a Taiwanese population. Neurobiol Aging 36:2905.e7–e8.

Chen M-K, Kuwabara H, Zhou Y, Adams RJ, Brašić JR, McGlothan JL, Verina T, Burton NC, Alexander M, Kumar A, Wong DF, Guilarte TR (2008) VMAT2 and dopamine neuron loss in a primate model of Parkinson's disease. J Neurochem 105:78–90.

Chen N, Reith ME (2000) Structure and function of the dopamine transporter. Eur J Pharmacol 405:329–339.

Chen S, Xu Y, Xu B, Guo M, Zhang Z, Liu L, Ma H, Chen Z, Luo Y, Huang S, Chen L (2011b) CaMKII is involved in cadmium activation of MAPK and mTOR pathways leading to neuronal cell death. J Neurochem 119:1108–1118.

Chen YA, Scheller RH (2001) SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol 2:98–106.

Cheon S-M, Chan L, Chan DKY, Kim JW (2012) Genetics of Parkinson's disease - a clinical perspective. J Mov Disord 5:33–41.

Cheung Y-T, Lau WK-W, Yu M-S, Lai CS-W, Yeung S-C, So K-F, Chang RC-C (2009) Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as in vitro model in neurotoxicity research. Neurotoxicology 30:127–135.

Chi L, Reith MEA (2003) Substrate-induced trafficking of the dopamine transporter in heterologously expressing cells and in rat striatal synaptosomal preparations. J Pharmacol Exp Ther 307:729–736.

Choi JC et al. (2015a) Effect of pre-stroke statin use on stroke severity and early functional recovery: a retrospective cohort study. BMC Neurol 15:120.

Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, Sulzer D, Mosharov EV (2015b) Changes in neuronal dopamine homeostasis following 1-methyl-4-phenylpyridinium (MPP+) exposure. J Biol Chem 290:6799–6809.

Chong PL-G, Zhu W, Venegas B (2009) On the lateral structure of model membranes containing cholesterol. Biochim Biophys Acta 1788:2–11.

Chouinard PA, Paus T (2006) The primary motor and premotor areas of the human cerebral cortex. Neurosci Rev J Bringing Neurobiol Neurol Psychiatry 12:143–152.

Ciccone S, Maiani E, Bellusci G, Diederich M, Gonfloni S (2013) Parkinson's disease: a complex interplay of mitochondrial DNA alterations and oxidative stress. Int J Mol Sci 14:2388–2409.

Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI (1995) The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci 15:1714–1723.

Clarke CE, Guttman M (2002) Dopamine agonist monotherapy in Parkinson's disease. Lancet Lond Engl 360:1767–1769.

Cohen G (1984) Oxy-radical toxicity in catecholamine neurons. Neurotoxicology 5:77–82.

Coleman M (2005) Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6:889–898.

Colgan SM, Tang D, Werstuck GH, Austin RC (2007) Endoplasmic reticulum stress causes the activation of sterol regulatory element binding protein-2. Int J Biochem Cell Biol 39:1843–1851.

Colliver TL, Pyott SJ, Achalabun M, Ewing AG (2000) VMAT-Mediated changes in quantal size and vesicular volume. J Neurosci Off J Soc Neurosci 20:5276–5282.

Connolly BS, Lang AE (2014) Pharmacological treatment of Parkinson disease: a review. JAMA 311:1670–1683.

Constantinescu R, Constantinescu AT, Reichmann H, Janetzky B (2007) Neuronal differentiation and long-term culture of the human neuroblastoma line SH-SY5Y. J Neural Transm Suppl:17–28.

Cook C, Stetler C, Petrucelli L (2012) Disruption of protein quality control in Parkinson's disease. Cold Spring Harb Perspect Med 2:a009423.

Cooper RA (1978) Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells. J Supramol Struct 8:413–430.

Copeland BJ, Vogelsberg V, Neff NH, Hadjiconstantinou M (1996) Protein kinase C activators decrease dopamine uptake into striatal synaptosomes. J Pharmacol Exp Ther 277:1527–1532.

Corasaniti MT, Defilippo R, Rodinò P, Nappi G, Nisticò G (1991) Evidence that paraquat is able to cross the blood-brain barrier to a different extent in rats of various age. Funct Neurol 6:385–391.

Corcoran J, Shroot B, Pizzey J, Maden M (2000) The role of retinoic acid receptors in neurite outgrowth from different populations of embryonic mouse dorsal root ganglia. J Cell Sci 113 ( Pt 14):2567–2574.

Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A (2010) Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. J Alzheimers Dis JAD 20 Suppl 1:S221–S238.

Cotzias GC, Papavasiliou PS, Gellene R (1969) Modification of Parkinsonism--chronic treatment with L-dopa. N Engl J Med 280:337–345.

Cotzias GC, Van Woert MH, Schiffer LM (1967) Aromatic amino acids and modification of parkinsonism. N Engl J Med 276:374–379.

Coune PG, Schneider BL, Aebischer P (2012) Parkinson's disease: gene therapies. Cold Spring Harb Perspect Med 2:a009431.

Cremona ML, Matthies HJG, Pau K, Bowton E, Speed N, Lute BJ, Anderson M, Sen N, Robertson SD, Vaughan RA, Rothman JE, Galli A, Javitch JA, Yamamoto A (2011) Flotillin-1 is essential for PKC-triggered endocytosis and membrane microdomain localization of DAT. Nat Neurosci 14:469–477.

Crèvecœur J, Foerch P, Doupagne M, Thielen C, Vandenplas C, Moonen G, Deprez M, Rogister B (2013) Expression of SV2 isoforms during rodent brain development. BMC Neurosci 14:87.

Cruz-Muros I, Afonso-Oramas D, Abreu P, Rodriguez M, Gonzalez MC, Gonzalez-Hernandez T (2008) Deglycosylation and subcellular redistribution of VMAT2 in the mesostriatal system during normal aging. Neurobiol Aging 29:1702–1711.

Dächsel JC, Behrouz B, Yue M, Beevers JE, Melrose HL, Farrer MJ (2010) A comparative study of Lrrk2 function in primary neuronal cultures. Parkinsonism Relat Disord 16:650–655.

Dahlstroem A, Fuxe K (1964) EVIDENCE FOR THE EXISTENCE OF MONOAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM. I. DEMONSTRATION OF MONOAMINES IN THE CELL BODIES OF BRAIN STEM NEURONS. Acta Physiol Scand Suppl:SUPPL 232:1–55.

Dai Y, Tan X, Wu W, Warner M, Gustafsson J-Å (2012) Liver X receptor β protects dopaminergic neurons in a mouse model of Parkinson disease. Proc Natl Acad Sci U S A 109:13112–13117.

Daniels GM, Amara SG (1999) Regulated trafficking of the human dopamine transporter. Clathrin-mediated internalization and lysosomal degradation in response to phorbol esters. J Biol Chem 274:35794–35801.

Dardou D, Dassesse D, Cuvelier L, Deprez T, De Ryck M, Schiffmann SN (2011) Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res 1367:130–145.

Dardou D, Monlezun S, Foerch P, Courade JP, Cuvelier L, De Ryck M, Schiffmann SN (2013) A role for Sv2c in basal ganglia functions. Brain Res 1507:61–73.

Datta SR, Brunet A, Greenberg ME (1999) Cellular survival: a play in three Akts. Genes Dev 13:2905–2927.

Daubner SC, Le T, Wang S (2011) Tyrosine hydroxylase and regulation of dopamine synthesis. Arch Biochem Biophys 508:1–12.

Davie CA (2008) A review of Parkinson's disease. Br Med Bull 86:109–127.

Decressac M, Volakakis N, Björklund A, Perlmann T (2013) NURR1 in Parkinson disease--from pathogenesis to therapeutic potential. Nat Rev Neurol 9:629–636.

de Lau LML, Breteler MMB (2006) Epidemiology of Parkinson's disease. Lancet Neurol 5:525–535.

DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.

Del Tredici K, Braak H (2013) Dysfunction of the locus coeruleus-norepinephrine system and related circuitry in Parkinson's disease-related dementia. J Neurol Neurosurg Psychiatry 84:774–783.

Delwaide PJ, Sabbatino M, Delwaide C (1986) Some pathophysiological aspects of the parkinsonian rigidity. J Neural Transm Suppl 22:129–139.

Deng H, Wu Y, Jankovic J (2015) The EIF4G1 gene and Parkinson's disease. Acta Neurol Scand 132:73–78.

Denker A, Kröhnert K, Bückers J, Neher E, Rizzoli SO (2011) The reserve pool of synaptic vesicles acts as a buffer for proteins involved in synaptic vesicle recycling. Proc Natl Acad Sci U S A 108:17183–17188.

Denker A, Rizzoli SO (2010) Synaptic vesicle pools: an update. Front Synaptic Neurosci 2:135.

De Rosa P, Marini ES, Gelmetti V, Valente EM (2015) Candidate genes for Parkinson disease: Lessons from pathogenesis. Clin Chim Acta Int J Clin Chem 449:68–76.

Dias V, Junn E, Mouradian MM (2013) The role of oxidative stress in Parkinson's disease. J Park Dis 3:461–491.

Dietschy JM (2009) Central nervous system: cholesterol turnover, brain development and neurodegeneration. Biol Chem 390:287–293.

Dietschy JM, Turley SD (2001) Cholesterol metabolism in the brain. Curr Opin Lipidol 12:105–112.

Dietschy JM, Turley SD (2004) Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 45:1375–1397.

DiFiglia M, Pasik P, Pasik T (1976) A Golgi study of neuronal types in the neostriatum of monkeys. Brain Res 114:245–256.

Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rütten H, Fichtlscherer S, Martin H, Zeiher AM (2001) HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J Clin Invest 108:391–397.

Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999) Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399:601–605.

Di Monte DA, Lavasani M, Manning-Bog AB (2002) Environmental factors in Parkinson's disease. Neurotoxicology 23:487–502.

Di-Poï N, Fauré J, Grizot S, Molnár G, Pick E, Dagher MC (2001) Mechanism of NADPH oxidase activation by the Rac/Rho-GDI complex. Biochemistry (Mosc) 40:10014–10022.

Dmetrichuk JM, Carlone RL, Spencer GE (2006) Retinoic acid induces neurite outgrowth and growth cone turning in invertebrate neurons. Dev Biol 294:39–49.

Do CB, Tung JY, Dorfman E, Kiefer AK, Drabant EM, Francke U, Mountain JL, Goldman SM, Tanner CM, Langston JW, Wojcicki A, Eriksson N (2011) Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. PLoS Genet 7:e1002141.

Dolga AM, Culmsee C, de Lau L, Winter Y, Oertel WH, Luiten PGM, Eisel ULM (2011) Statins--increasing or reducing the risk of Parkinson's disease? Exp Neurol 228:1–4.

Dolga AM, Granic I, Nijholt IM, Nyakas C, van der Zee EA, Luiten PGM, Eisel ULM (2009) Pretreatment with lovastatin prevents N-methyl-D-aspartate-induced neurodegeneration in the magnocellular nucleus basalis and behavioral dysfunction. J Alzheimers Dis JAD 17:327–336.

Dolga AM, Nijholt IM, Ostroveanu A, Ten Bosch Q, Luiten PGM, Eisel ULM (2008) Lovastatin induces neuroprotection through tumor necrosis factor receptor 2 signaling pathways. J Alzheimers Dis JAD 13:111–122.

Domanskyi A, Alter H, Vogt MA, Gass P, Vinnikov IA (2014) Transcription factors Foxa1 and Foxa2 are required for adult dopamine neurons maintenance. Front Cell Neurosci 8:275.

Dong W, Vuletic S, Albers JJ (2009) Differential effects of simvastatin and pravastatin on expression of Alzheimer's disease-related genes in human astrocytes and neuronal cells. J Lipid Res 50:2095–2102.

Donovan DM, Miner LL, Perry MP, Revay RS, Sharpe LG, Przedborski S, Kostic V, Philpot RM, Kirstein CL, Rothman RB, Schindler CW, Uhl GR (1999) Cocaine reward and MPTP toxicity: alteration by regional variant dopamine transporter overexpression. Brain Res Mol Brain Res 73:37–49.

Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, Marshall FJ, Ravina BM, Schifitto G, Siderowf A, Tanner CM (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 68:384–386.

Douglas MR (2013) Gene therapy for Parkinson's disease: state-of-the-art treatments for neurodegenerative disease. Expert Rev Neurother 13:695–705.

Dusek P, Roos PM, Litwin T, Schneider SA, Flaten TP, Aaseth J (2015) The neurotoxicity of iron, copper and manganese in Parkinson's and Wilson's diseases. J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS 31:193–203.

Duty S, Jenner P (2011) Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol 164:1357–1391.

Dwane S, Durack E, Kiely PA (2013) Optimising parameters for the differentiation of SH-SY5Y cells to study cell adhesion and cell migration. BMC Res Notes 6:366.

Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F (2004) SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86:839–848.

Edmond J, Korsak RA, Morrow JW, Torok-Both G, Catlin DH (1991) Dietary cholesterol and the origin of cholesterol in the brain of developing rats. J Nutr 121:1323–1330.

Edmondson DE, Mattevi A, Binda C, Li M, Hubálek F (2004) Structure and mechanism of monoamine oxidase. Curr Med Chem 11:1983–1993.

Edvardson S, Cinnamon Y, Ta-Shma A, Shaag A, Yim Y-I, Zenvirt S, Jalas C, Lesage S, Brice A, Taraboulos A, Kaestner KH, Greene LE, Elpeleg O (2012) A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PloS One 7:e36458.

Edwards PA, Tabor D, Kast HR, Venkateswaran A (2000) Regulation of gene expression by SREBP and SCAP. Biochim Biophys Acta 1529:103–113.

Egana LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Pena K, Quiroz M, Hong WC, Dorostkar MM, Janz R, Sitte HH, Torres GE (2009) Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. J Neurosci 29:4592–4604.

Ehringer H, Hornykiewicz O (1960) [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. Klin Wochenschr 38:1236–1239.

Eiden LE, Sch&#65533;fer MK-H, Weihe E, Sch&#65533;tz B (2004) The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. Pfl&#65533;gers Arch Eur J Physiol 447:636–640.

Elsworth JD, Roth RH (1997) Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol 144:4–9.

Encinas M, Iglesias M, Liu Y, Wang H, Muhaisen A, Ceña V, Gallego C, Comella JX (2000) Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. J Neurochem 75:991–1003.

Endo A, Kuroda M, Tsujita Y (1976) ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinium. J Antibiot (Tokyo) 29:1346–1348.

Engelking LJ, Liang G, Hammer RE, Takaishi K, Kuriyama H, Evers BM, Li W-P, Horton JD, Goldstein JL, Brown MS (2005) Schoenheimer effect explained--feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. J Clin Invest 115:2489–2498.

Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E (1996) Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. Proc Natl Acad Sci U S A 93:5166–5171.

Erickson JD, Varoqui H (2000) Molecular analysis of vesicular amine transporter function and targeting to secretory organelles. FASEB J Off Publ Fed Am Soc Exp Biol 14:2450–2458.

Eriksen J, Jørgensen TN, Gether U (2010) Regulation of dopamine transporter function by protein-protein interactions: new discoveries and methodological challenges. J Neurochem 113:27–41.

Erro R (2015) VPS35 and EIF4G1 interactions and novel candidate genes for PD: from genes to pathways and back. Mov Disord Off J Mov Disord Soc 30:499.

Espenshade PJ (2006) SREBPs: sterol-regulated transcription factors. J Cell Sci 119:973–976.

Esteves AR, Arduíno DM, Swerdlow RH, Oliveira CR, Cardoso SM (2009) Oxidative stress involvement in alpha-synuclein oligomerization in Parkinson's disease cybrids. Antioxid Redox Signal 11:439–448.

Esteves M, Cristóvão AC, Saraiva T, Rocha SM, Baltazar G, Ferreira L, Bernardino L (2015) Retinoic acid-loaded polymeric nanoparticles induce neuroprotection in a mouse model for Parkinson's disease. Front Aging Neurosci 7:20.

Evangelopoulos ME, Weis J, Kruttgen A (2009) Mevastatin-induced neurite outgrowth of neuroblastoma cells via activation of EGFR. J Neurosci Res 87:2138–2144.

Fahn S (2008) The history of dopamine and levodopa in the treatment of Parkinson's disease. Mov Disord Off J Mov Disord Soc 23 Suppl 3:S497–S508.

Fantini J, Yahi N (2015) Brain lipids in synaptic function and neurological disease: clues to innovative therapeutic strategies for brain disorders. Available at: http://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=992468 [Accessed September 17, 2015].

Farrar NR, Dmetrichuk JM, Carlone RL, Spencer GE (2009) A novel, nongenomic mechanism underlies retinoic acid-induced growth cone turning. J Neurosci Off J Soc Neurosci 29:14136–14142.

Fathi F, Altiraihi T, Mowla SJ, Movahedin M (2010) Transplantation of retinoic acid treated murine embryonic stem cells & behavioural deficit in Parkinsonian rats. Indian J Med Res 131:536–544.

Fei H, Grygoruk A, Brooks ES, Chen A, Krantz DE (2008) Trafficking of vesicular neurotransmitter transporters. Traffic Cph Den 9:1425–1436.

Fernández-Hernando C, Suárez Y, Lasunción MA (2005) Lovastatin-induced PC-12 cell differentiation is associated with RhoA/RhoA kinase pathway inactivation. Mol Cell Neurosci 29:591–602.

Ferré P, Foufelle F (2007) SREBP-1c transcription factor and lipid homeostasis: clinical perspective. Horm Res 68:72–82.

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681–698.

Floor E, Feist BE (1989) Most synaptic vesicles isolated from rat brain carry three membrane proteins, SV2, synaptophysin, and p65. J Neurochem 52:1433–1437.

Floor E, Meng L (1996) Amphetamine releases dopamine from synaptic vesicles by dual mechanisms. Neurosci Lett 215:53–56.

Floor E, Schaeffer SF, Feist BE, Leeman SE (1988) Synaptic vesicles from mammalian brain: large-scale purification and physical and immunochemical characterization. J Neurochem 50:1588–1596.

Fon EA, Pothos EN, Sun BC, Killeen N, Sulzer D, Edwards RH (1997) Vesicular transport regulates monoamine storage and release but is not essential for amphetamine action. Neuron 19:1271–1283.

Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH (2004) Lipid rafts mediate the synaptic localization of alpha-synuclein. J Neurosci Off J Soc Neurosci 24:6715–6723.

Foster HD, Hoffer A (2004) The two faces of L-DOPA: benefits and adverse side effects in the treatment of Encephalitis lethargica, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis. Med Hypotheses 62:177–181.

Foster JD, Adkins SD, Lever JR, Vaughan RA (2008) Phorbol ester induced trafficking-independent regulation and enhanced phosphorylation of the dopamine transporter associated with membrane rafts and cholesterol. J Neurochem 105:1683–1699.

Fountaine TM, Wade-Martins R (2007) RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP(+) toxicity and reduces dopamine transport. J Neurosci Res 85:351–363.

Fox SH, Chuang R, Brotchie JM (2009) Serotonin and Parkinson's disease: On movement, mood, and madness. Mov Disord Off J Mov Disord Soc 24:1255–1266.

Freed C, Revay R, Vaughan RA, Kriek E, Grant S, Uhl GR, Kuhar MJ (1995) Dopamine transporter immunoreactivity in rat brain. J Comp Neurol 359:340–349.

Freed CR, Zhou W, Breeze RE (2011) Dopamine cell transplantation for Parkinson's disease: the importance of controlled clinical trials. Neurother J Am Soc Exp Neurother 8:549–561.

Friesen JA, Rodwell VW (2004) The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases. Genome Biol 5:248.

Friling S, Bergsland M, Kjellander S (2009) Activation of Retinoid X Receptor increases dopamine cell survival in models for Parkinson's disease. BMC Neurosci 10:146.

Fujioka S, Sundal C, Strongosky AJ, Castanedes MC, Rademakers R, Ross OA, Vilariño-Güell C, Farrer MJ, Wszolek ZK, Dickson DW (2013) Sequence variants in eukaryotic translation initiation factor 4-gamma (eIF4G1) are associated with Lewy body dementia. Acta Neuropathol (Berl) 125:425–438.

Fujioka S, Wszolek ZK (2012) Update on genetics of parkinsonism. Neurodegener Dis 10:257–260.

Fujita Y, Yamashita T (2014) Axon growth inhibition by RhoA/ROCK in the central nervous system. Front Neurosci 8:338.

Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC (1999) Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 399:597–601.

Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, Chesebro JH (1990) Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 82:II47–II59.

Fuxe K, Manger P, Genedani S, Agnati L (2006) The nigrostriatal DA pathway and Parkinson's disease. J Neural Transm Suppl:71–83.

Gabbi C, Warner M, Gustafsson J-Å (2014) Action mechanisms of Liver X Receptors. Biochem Biophys Res Commun 446:647–650.

Gainetdinov RR, Fumagalli F, Jones SR, Caron MG (1997) Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter. J Neurochem 69:1322–1325.

Gainetdinov RR, Fumagalli F, Wang Y-M, Jones SR, Levey AI, Miller GW, Caron MG (1998) Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. J Neurochem 70:1973–1978.

Gao X, Simon KC, Schwarzschild MA, Ascherio A (2012) Prospective study of statin use and risk of Parkinson disease. Arch Neurol 69:380–384.

Garcia BG, Wei Y, Moron JA, Lin RZ, Javitch JA, Galli A (2005) Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. Mol Pharmacol 68:102–109.

Gasic GP (1994) Basic-helix-loop-helix transcription factor and sterol sensor in a single membrane-bound molecule. Cell 77:17–19.

Gaven F, Marin P, Claeysen S (2014) Primary culture of mouse dopaminergic neurons. J Vis Exp JoVE:e51751.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.

Gershanik OS (2015) Improving L-dopa therapy: the development of enzyme inhibitors. Mov Disord Off J Mov Disord Soc 30:103–113.

Gessner W, Brossi A, Shen RS, Abell CW (1985) Further insight into the mode of action of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). FEBS Lett 183:345–348.

Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, Pahan K (2009) Simvastatin inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson's disease. J Neurosci 29:13543–13556.

Giugni JC, Okun MS (2014) Treatment of advanced Parkinson's disease: Curr Opin Neurol 27:450–460.

Glatt CE, Wahner AD, White DJ, Ruiz-Linares A, Ritz B (2006) Gain-of-function haplotypes in the vesicular monoamine transporter promoter are protective for Parkinson disease in women. Hum Mol Genet 15:299–305.

Goedert M (1999) Filamentous nerve cell inclusions in neurodegenerative diseases: tauopathies and alpha-synucleinopathies. Philos Trans R Soc Lond B Biol Sci 354:1101–1118.

Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, Giladi N, Holloway RG, Moore CG, Wenning GK, Yahr MD, Seidl L, Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease (2004) Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord Off J Mov Disord Soc 19:1020–1028.

Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E (2004) Parkinsonism among Gaucher disease carriers. J Med Genet 41:937–940.

Goldman SM (2014) Environmental toxins and Parkinson's disease. Annu Rev Pharmacol Toxicol 54:141–164.

Goldman SM, Kamel F, Ross GW, Jewell SA, Bhudhikanok GS, Umbach D, Marras C, Hauser RA, Jankovic J, Factor SA, Bressman S, Lyons KE, Meng C, Korell M, Roucoux DF, Hoppin JA, Sandler DP, Langston JW, Tanner CM (2012) Head injury, α-synuclein Rep1, and Parkinson's disease. Ann Neurol 71:40–48.

Goldstein JL, Brown MS (1990) Regulation of the mevalonate pathway. Nature 343:425–430.

Goldstein JL, Brown MS (2009) The LDL receptor. Arterioscler Thromb Vasc Biol 29:431–438.

González-Hernández T, Barroso-Chinea P, De La Cruz Muros I, Del Mar Pérez-Delgado M, Rodríguez M (2004) Expression of dopamine and vesicular monoamine transporters and differential vulnerability of mesostriatal dopaminergic neurons. J Comp Neurol 479:198–215.

González-Hernández T, Cruz-Muros I, Afonso-Oramas D, Salas-Hernandez J, Castro-Hernandez J (2010) Vulnerability of mesostriatal dopaminergic neurons in Parkinson's disease. Front Neuroanat 4:140.

Goodman CS (1996) Mechanisms and molecules that control growth cone guidance. Annu Rev Neurosci 19:341–377.

Graham DG, Tiffany SM, Bell WR, Gutknecht WF (1978) Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. Mol Pharmacol 14:644–653.

Grattan DR (2015) 60 YEARS OF NEUROENDOCRINOLOGY: The hypothalamo-prolactin axis. J Endocrinol 226:T101–T122.

Greenwood J, Steinman L, Zamvil SS (2006) Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. Nat Rev Immunol 6:358–370.

Greggio E (2012) Role of LRRK2 kinase activity in the pathogenesis of Parkinson's disease. Biochem Soc Trans 40:1058–1062.

Guillot TS, Miller GW (2009) Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. Mol Neurobiol 39:149–170.

Guo JT, Chen AQ, Kong Q, Zhu H, Ma CM, Qin C (2008) Inhibition of vesicular monoamine transporter-2 activity in alpha-synuclein stably transfected SH-SY5Y cells. Cell Mol Neurobiol 28:35–47.

Guo X, Song W, Chen K, Chen X, Zheng Z, Cao B, Huang R, Zhao B, Wu Y, Shang H-F (2014) The serum lipid profile of Parkinson's disease patients: a study from China. Int J Neurosci.

Gupta A, Dey CS (2012) PTEN, a widely known negative regulator of insulin/PI3K signaling, positively regulates neuronal insulin resistance. Mol Biol Cell 23:3882–3898.

Gustavsson A et al. (2011) Cost of disorders of the brain in Europe 2010. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol 21:718–779.

Gutman Y, Segal J (1972) Effect of calcium, sodium and potassium on adrenal tyrosine hydroxylase activity in vitro. Biochem Pharmacol 21:2664–2666.

Haber SN (2014) The place of dopamine in the cortico-basal ganglia circuit. Neuroscience 282:248–257.

Häbig K, Gellhaar S, Heim B, Djuric V, Giesert F, Wurst W, Walter C, Hentrich T, Riess O, Bonin M (2013) LRRK2 guides the actin cytoskeleton at growth cones together with ARHGEF7 and Tropomyosin 4. Biochim Biophys Acta 1832:2352–2367.

Halliday GM, Blumbergs PC, Cotton RG, Blessing WW, Geffen LB (1990) Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. Brain Res 510:104–107.

Hanganu A, Bedetti C, Jubault T, Gagnon J-F, Mejia-Constain B, Degroot C, Lafontaine A-L, Chouinard S, Monchi O (2013) Mild cognitive impairment in patients with Parkinson's disease is associated with increased cortical degeneration. Mov Disord Off J Mov Disord Soc 28:1360–1369.

Härmälä AS, Pörn MI, Mattjus P, Slotte JP (1994) Cholesterol transport from plasma membranes to intracellular membranes is inhibited by 3 beta-[2-(diethylamino)ethoxy]androst-5-en-17-one. Biochim Biophys Acta 1211:317–325.

Harris KM, Jensen FE, Tsao B (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci Off J Soc Neurosci 12:2685–2705.

Harris KM, Weinberg RJ (2012) Ultrastructure of synapses in the mammalian brain. Cold Spring Harb Perspect Biol 4.

Hashemi SH, Li J-Y, Ahlman H, Dahlström A (2003) SSR2(a) receptor expression and adrenergic/cholinergic characteristics in differentiated SH-SY5Y cells. Neurochem Res 28:449–460.

Hassler R (1938) Zur pathologie der paralysis agitans und des postencephalitschen Parkinsonismus. J Psychol Neurol 48:387–476.

Hastings TG, Lewis DA, Zigmond MJ (1996a) Reactive dopamine metabolites and neurotoxicity: implications for Parkinson's disease. Adv Exp Med Biol 387:97–106.

Hastings TG, Lewis DA, Zigmond MJ (1996b) Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. Proc Natl Acad Sci U S A 93:1956–1961.

Hastrup H, Karlin A, Javitch JA (2001) Symmetrical dimer of the human dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment. Proc Natl Acad Sci U S A 98:10055–10060.

Hastrup H, Sen N, Javitch JA (2003) The human dopamine transporter forms a tetramer in the plasma membrane: cross-linking of a cysteine in the fourth transmembrane segment is sensitive to cocaine analogs. J Biol Chem 278:45045–45048.

Hauser RA (2009) Levodopa: past, present, and future. Eur Neurol 62:1–8.

Hawkins JL, Robbins MD, Warren LC, Xia D, Petras SF, Valentine JJ, Varghese AH, Wang I-K, Subashi TA, Shelly LD, Hay BA, Landschulz KT, Geoghegan KF, Harwood HJ (2008) Pharmacologic inhibition of site 1 protease activity inhibits sterol regulatory element-binding protein processing and reduces lipogenic enzyme gene expression and lipid synthesis in cultured cells and experimental animals. J Pharmacol Exp Ther 326:801–808.

Hay BA, Abrams B, Zumbrunn AY, Valentine JJ, Warren LC, Petras SF, Shelly LD, Xia A, Varghese AH, Hawkins JL, Van Camp JA, Robbins MD, Landschulz K, Harwood HJ (2007) Aminopyrrolidineamide inhibitors of site-1 protease. Bioorg Med Chem Lett 17:4411–4414.

Hegarty SV, O'Keeffe GW, Sullivan AM (2014) Neurotrophic factors: from neurodevelopmental regulators to novel therapies for Parkinson's disease. Neural Regen Res 9:1708–1711.

He L, Lu J, Zhou Q, Sui S (2006) Lipid Rafts Identified on Synaptic Vesicles from Rat Brain*. Tsinghua Sci Technol 11:452–458.

Hemmings BA, Restuccia DF (2012) PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol 4:a011189.

Henchcliffe C, Severt WL (2011) Disease modification in Parkinson's disease. Drugs Aging 28:605–615.

Heo HY, Kim K-S, Seol W (2010) Coordinate Regulation of Neurite Outgrowth by LRRK2 and Its Interactor, Rab5. Exp Neurobiol 19:97–105.

Herkenham M, Little MD, Bankiewicz K, Yang SC, Markey SP, Johannessen JN (1991) Selective retention of MPP+ within the monoaminergic systems of the primate brain following MPTP administration: an in vivo autoradiographic study. Neuroscience 40:133–158.

Hermanson E, Borgius L, Bergsland M, Joodmardi E, Perlmann T (2006) Neuropilin1 is a direct downstream target of Nurr1 in the developing brain stem. J Neurochem 97:1403–1411.

Hermanson E, Joseph B, Castro D, Lindqvist E, Aarnisalo P, Wallén A, Benoit G, Hengerer B, Olson L, Perlmann T (2003) Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. Exp Cell Res 288:324–334.

Hernández-Perera O, Pérez-Sala D, Navarro-Antolín J, Sánchez-Pascuala R, Hernández G, Díaz C, Lamas S (1998) Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. J Clin Invest 101:2711–2719.

Hernández-Perera O, Pérez-Sala D, Soria E, Lamas S (2000) Involvement of Rho GTPases in the transcriptional inhibition of preproendothelin-1 gene expression by simvastatin in vascular endothelial cells. Circ Res 87:616–622.

Higuchi M, Onishi K, Masuyama N, Gotoh Y (2003) The phosphatidylinositol-3 kinase (PI3K)-Akt pathway suppresses neurite branch formation in NGF-treated PC12 cells. Genes Cells Devoted Mol Cell Mech 8:657–669.

Hill-Burns EM, Singh N, Ganguly P, Hamza TH, Montimurro J, Kay DM, Yearout D, Sheehan P, Frodey K, McLear JA, Feany MB, Hanes SD, Wolfgang WJ, Zabetian CP, Factor SA, Payami H (2013) A genetic basis for the variable effect of smoking/nicotine on Parkinson's disease. Pharmacogenomics J 13:530–537.

Hirata Y, Suzuno H, Tsuruta T, Oh-hashi K, Kiuchi K (2008) The role of dopamine transporter in selective toxicity of manganese and rotenone. Toxicology 244:249–256.

Hirsch EC, Hunot S (2009) Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol 8:382–397.

Hirsch EC, Vyas S, Hunot S (2012) Neuroinflammation in Parkinson's disease. Parkinsonism Relat Disord 18 Suppl 1:S210–S212.

Hoehn MM, Yahr MD (1967) Parkinsonism: onset, progression and mortality. Neurology 17:427–442.

Holstein SA, Hohl RJ (2004) Isoprenoids: remarkable diversity of form and function. Lipids 39:293–309.

Höltje M, Winter S, Walther D, Pahner I, Hörtnagl H, Ottersen OP, Bader M, Ahnert-Hilger G (2003) The vesicular monoamine content regulates VMAT2 activity through Galphaq in mouse platelets. Evidence for autoregulation of vesicular transmitter uptake. J Biol Chem 278:15850–15858.

Holtz P, Heise R, Lüdtke K (1938) Fermentativer Abbau von l-Dioxyphenylalanin (Dopa) durch Niere. Naunyn-Schmiedebergs Arch Für Exp Pathol Pharmakol 191:87–118.

Hong S, Chung S, Leung K, Hwang I, Moon J, Kim K-S (2014) Functional roles of Nurr1, Pitx3, and Lmx1a in neurogenesis and phenotype specification of dopamine neurons during in vitro differentiation of embryonic stem cells. Stem Cells Dev 23:477–487.

Hooff GP, Wood WG, Müller WE, Eckert GP (2010) Isoprenoids, small GTPases and Alzheimer's disease. Biochim Biophys Acta 1801:896–905.

Horowski R (2007) A history of dopamine agonists. From the physiology and pharmacology of dopamine to therapies for prolactinomas and Parkinson's disease - a subjective view. J Neural Transm Vienna Austria 1996 114:127–134.

Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131.

Houlden H, Singleton AB (2012) The genetics and neuropathology of Parkinson's disease. Acta Neuropathol (Berl) 124:325–338.

Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, Kirchgessner TG (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem 274:37161–37168.

Huang X, Abbott RD, Petrovitch H, Mailman RB, Ross GW (2008) Low LDL cholesterol and increased risk of Parkinson's disease: prospective results from Honolulu-Asia Aging Study. Mov Disord Off J Mov Disord Soc 23:1013–1018.

Huang X, Chen H, Miller WC, Mailman RB, Woodard JL, Chen PC, Xiang D, Murrow RW, Wang Y-Z, Poole C (2007) Lower low-density lipoprotein cholesterol levels are associated with Parkinson's disease. Mov Disord Off J Mov Disord Soc 22:377–381.

Hu G, Antikainen R, Jousilahti P, Kivipelto M, Tuomilehto J (2008) Total cholesterol and the risk of Parkinson disease. Neurology 70:1972–1979.

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55:181–184.

Hughes M, Snetkov V, Rose R-S, Trousil S, Mermoud JE, Dingwall C (2010) Neurite-like structures induced by mevalonate pathway blockade are due to the stability of cell adhesion foci and are enhanced by the presence of APP. J Neurochem 114:832–842.

Hunot S, Boissière F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, Hirsch EC (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. Neuroscience 72:355–363.

Hunot S, Brugg B, Ricard D, Michel PP, Muriel MP, Ruberg M, Faucheux BA, Agid Y, Hirsch EC (1997) Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with parkinson disease. Proc Natl Acad Sci U S A 94:7531–7536.

Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debré P, Agid Y, Dugas B, Hirsch EC (1999) FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. J Neurosci Off J Soc Neurosci 19:3440–3447.

Hunot S, Hirsch EC (2003) Neuroinflammatory processes in Parkinson's disease. Ann Neurol 53 Suppl 3:S49–S58; discussion S58–S60.

Huot P, Fox SH, Brotchie JM (2015) Monoamine reuptake inhibitors in Parkinson's disease. Park Dis 2015:609428.

Huse DM, Schulman K, Orsini L, Castelli-Haley J, Kennedy S, Lenhart G (2005) Burden of illness in Parkinson's disease. Mov Disord Off J Mov Disord Soc 20:1449–1454.

Hwang D-Y, Hong S, Jeong J-W, Choi S, Kim H, Kim J, Kim K-S (2009) Vesicular monoamine transporter 2 and dopamine transporter are molecular targets of Pitx3 in the ventral midbrain dopamine neurons. J Neurochem 111:1202–1212.

Hynds DL, Spencer ML, Andres DA, Snow DM (2003) Rit promotes MEK-independent neurite branching in human neuroblastoma cells. J Cell Sci 116:1925–1935.

Ichikawa M, Yoshida J, Saito K, Sagawa H, Tokita Y, Watanabe M (2008) Differential effects of two ROCK inhibitors, Fasudil and Y-27632, on optic nerve regeneration in adult cats. Brain Res 1201:23–33.

Ikonen E (2008) Cellular cholesterol trafficking and compartmentalization. Nat Rev Mol Cell Biol 9:125–138.

Illingworth DR, Tobert JA (1994) A review of clinical trials comparing HMG-CoA reductase inhibitors. Clin Ther 16:366–385; discussion 365.

Istvan ES, Deisenhofer J (2001) Structural mechanism for statin inhibition of HMG-CoA reductase. Science 292:1160–1164.

Ivatt RM, Sanchez-Martinez A, Godena VK, Brown S, Ziviani E, Whitworth AJ (2014) Genome-wide RNAi screen identifies the Parkinson disease GWAS risk locus SREBF1 as a regulator of mitophagy. Proc Natl Acad Sci U S A 111:8494–8499.

Ivatt RM, Whitworth AJ (2014) SREBF1 links lipogenesis to mitophagy and sporadic Parkinson disease. Autophagy 10:1476–1477.

Jaber M, Robinson SW, Missale C, Caron MG (1996) Dopamine receptors and brain function. Neuropharmacology 35:1503–1519.

Jackson MB (2010) SNARE complex zipping as a driving force in the dilation of proteinaceous fusion pores. J Membr Biol 235:89–100.

Jacobs FMJ, van Erp S, van der Linden AJA, von Oerthel L, Burbach JPH, Smidt MP (2009) Pitx3 potentiates Nurr1 in dopamine neuron terminal differentiation through release of SMRT-mediated repression. Dev Camb Engl 136:531–540.

Jacobs FMJ, Veenvliet JV, Almirza WH, Hoekstra EJ, von Oerthel L, van der Linden AJA, Neijts R, Koerkamp MG, van Leenen D, Holstege FCP, Burbach JPH, Smidt MP (2011) Retinoic acid-dependent and -independent gene-regulatory pathways of Pitx3 in meso-diencephalic dopaminergic neurons. Dev Camb Engl 138:5213–5222.

Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. Nature 490:201–207.

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–376.

Jankovic J, Aguilar LG (2008) Current approaches to the treatment of Parkinson's disease. Neuropsychiatr Dis Treat 4:743–757.

Jankovic J, Tolosa E (2015) Parkinson's disease & movement disorders.

Janowsky A, Neve K, Eshleman AJ (2001) Uptake and release of neurotransmitters. Curr Protoc Neurosci Chapter 7:Unit7 9.

Janz R, Goda Y, Geppert M, Missler M, Südhof TC (1999) SV2A and SV2B function as redundant Ca2+ regulators in neurotransmitter release. Neuron 24:1003–1016.

Janz R, Hofmann K, Südhof TC (1998) SVOP, an evolutionarily conserved synaptic vesicle protein, suggests novel transport functions of synaptic vesicles. J Neurosci Off J Soc Neurosci 18:9269–9281.

Janz R, Südhof TC (1999) SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. Neuroscience 94:1279–1290.

Jassen AK, Brown JM, Panas HN, Miller GM, Xiao D, Madras BK (2005) Variants of the primate vesicular monoamine transporter-2. Brain Res Mol Brain Res 139:251–257.

Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. Proc Natl Acad Sci U S A 82:2173–2177.

Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol Spons Int Soc Neurochem World Fed Neurol Res Groups Neurochem Cerebrospinal Fluid 14:153–197.

Jellinger KA (1999) Post mortem studies in Parkinson's disease--is it possible to detect brain areas for specific symptoms? J Neural Transm Suppl 56:1–29.

Jenner P, Olanow CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47:S161–S170.

Jeon T-I, Osborne TF (2012) SREBPs: metabolic integrators in physiology and metabolism. Trends Endocrinol Metab TEM 23:65–72.

Jha SK, Jha NK, Kar R, Ambasta RK, Kumar P (2015) p38 MAPK and PI3K/AKT Signalling Cascades inParkinson's Disease. Int J Mol Cell Med 4:67–86.

Jiang C, Wan X, He Y, Pan T, Jankovic J, Le W (2005) Age-dependent dopaminergic dysfunction in Nurr1 knockout mice. Exp Neurol 191:154–162.

Jiang H, Jiang Q, Feng J (2004) Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. J Biol Chem 279:54380–54386.

Jin Y, Sui HJ, Dong Y, Ding Q, Qu WH, Yu SX, Jin YX (2012) Atorvastatin enhances neurite outgrowth in cortical neurons in vitro via up-regulating the Akt/mTOR and Akt/GSK-3beta signaling pathways. Acta Pharmacol Sin 33:861–872.

Johnson-Anuna LN, Eckert GP, Keller JH, Igbavboa U, Franke C, Fechner T, Schubert-Zsilavecz M, Karas M, Muller WE, Wood WG (2005) Chronic administration of statins alters multiple gene expression patterns in mouse cerebral cortex. J Pharmacol Exp Ther 312:786–793.

Jones KT, Zhen J, Reith MEA (2012) Importance of cholesterol in dopamine transporter function. J Neurochem 123:700–715.

Jones PH (2003) Comparing HMG-CoA reductase inhibitors. Clin Cardiol 26:I15–I20.

Jo Y, Debose-Boyd RA (2010) Control of cholesterol synthesis through regulated ER-associated degradation of HMG CoA reductase. Crit Rev Biochem Mol Biol 45:185–198.

Kadkhodaei B, Ito T, Joodmardi E, Mattsson B, Rouillard C, Carta M, Muramatsu S, Sumi-Ichinose C, Nomura T, Metzger D, Chambon P, Lindqvist E, Larsson N-G, Olson L, Björklund A, Ichinose H, Perlmann T (2009) Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. J Neurosci Off J Soc Neurosci 29:15923–15932.

Kalia LV, Kalia SK, Lang AE (2015) Disease-modifying strategies for Parkinson's disease. Mov Disord Off J Mov Disord Soc 30:1442–1450.

Kalia LV, Lang AE (2015) Parkinson's disease. Lancet.

Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferre P, Heldin CH, Delzenne NM, Demoulin JB (2007a) SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 gamma. J Lipid Res 48:1628–1636.

Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferre P, Heldin CH, Delzenne NM, Demoulin JB (2007b) SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 gamma. J Lipid Res 48:1628–1636.

Kamishibahara Y, Kawaguchi H, Shimizu N (2014) Promotion of mouse embryonic stem cell differentiation by Rho kinase inhibitor Y-27632. Neurosci Lett 579:58–63.

Kastner A, Hirsch EC, Lejeune O, Javoy-Agid F, Rascol O, Agid Y (1992) Is the vulnerability of neurons in the substantia nigra of patients with Parkinson's disease related to their neuromelanin content? J Neurochem 59:1080–1089.

Katzenschlager R, Sampaio C, Costa J, Lees A (2003) Anticholinergics for symptomatic management of Parkinson's disease. Cochrane Database Syst Rev:CD003735.

Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.

Kawakami F, Yabata T, Ohta E, Maekawa T, Shimada N, Suzuki M, Maruyama H, Ichikawa T, Obata F (2012) LRRK2 phosphorylates tubulin-associated tau but not the free molecule: LRRK2-mediated regulation of the tau-tubulin association and neurite outgrowth. PloS One 7:e30834.

Keller JN, Gee J, Ding Q (2002) The proteasome in brain aging. Ageing Res Rev 1:279–293.

Kempster PA, Wahlqvist ML (1994) Dietary factors in the management of Parkinson's disease. Nutr Rev 52:51–58.

Kerstein PC, Nichol RH, Gomez TM (2015) Mechanochemical regulation of growth cone motility. Front Cell Neurosci 9:244.

Kim JB, Spiegelman BM (1996) ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev 10:1096–1107.

Kim K-S, Kang Y-M, Kang Y, Park T-S, Park H-Y, Kim Y-J, Han B-S, Kim C-H, Lee C-H, Ardayfio PA, Han P-L, Jung B-H, Kim K-S (2014) Pitx3 deficient mice as a genetic animal model of co-morbid depressive disorder and parkinsonism. Brain Res 1552:72–81.

Kim K-S, Kim C-H, Hwang D-Y, Seo H, Chung S, Hong SJ, Lim J-K, Anderson T, Isacson O (2003) Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. J Neurochem 85:622–634.

Kim Y, Chang S (2004) Modulation of actomyosin contractility by myosin light chain phosphorylation/dephosphorylation through Rho GTPases signaling specifies axon formation in neurons. Biochem Biophys Res Commun 318:579–587.

Kintscher U (2012) Reuptake inhibitors of dopamine, noradrenaline, and serotonin. Handb Exp Pharmacol:339–347.

Kirshner N (1962) Uptake of catecholamines by a particulate fraction of the adrenal medulla. Science 135:107–108.

Kish SJ, Shannak K, Hornykiewicz O (1988) Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med 318:876–880.

Kitagishi Y, Kobayashi M, Kikuta K, Matsuda S (2012) Roles of PI3K/AKT/GSK3/mTOR Pathway in Cell Signaling of Mental Illnesses. Depress Res Treat 2012:752563.

Kitamura M, Ishikawa Y, Moreno-Manzano V, Xu Q, Konta T, Lucio-Cazana J, Furusu A, Nakayama K (2002) Intervention by retinoic acid in oxidative stress-induced apoptosis. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc 17 Suppl 9:84–87.

Kitayama S, Mitsuhata C, Davis S, Wang JB, Sato T, Morita K, Uhl GR, Dohi T (1998) MPP+ toxicity and plasma membrane dopamine transporter: study using cell lines expressing the wild-type and mutant rat dopamine transporters. Biochim Biophys Acta 1404:305–313.

Kittappa R, Chang WW, Awatramani RB, McKay RDG (2007) The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol 5:e325.

Klaidman LK, Adams JD, Leung AC, Kim SS, Cadenas E (1993) Redox cycling of MPP+: evidence for a new mechanism involving hydride transfer with xanthine oxidase, aldehyde dehydrogenase, and lipoamide dehydrogenase. Free Radic Biol Med 15:169–179.

Klein C, Westenberger A (2012) Genetics of Parkinson's Disease. Cold Spring Harb Perspect Med 2:a008888–a008888.

Knoth J, Zallakian M, Njus D (1981) Stoichiometry of H+-linked dopamine transport in chromaffin granule ghosts. Biochemistry (Mosc) 20:6625–6629.

Knott C, Stern G, Wilkin GP (2000) Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci 16:724–739.

Kobilka BK (2007) G protein coupled receptor structure and activation. Biochim Biophys Acta 1768:794–807.

Koch JC, Bitow F, Haack J, d'Hedouville Z, Zhang J-N, Tönges L, Michel U, Oliveira LMA, Jovin TM, Liman J, Tatenhorst L, Bähr M, Lingor P (2015) Alpha-Synuclein affects neurite morphology, autophagy, vesicle transport and axonal degeneration in CNS neurons. Cell Death Dis 6:e1811.

Koller WC, Montgomery EB (1997) Issues in the early diagnosis of Parkinson's disease. Neurology 49:S10–S25.

Koob AO, Ubhi K, Paulsson JF, Kelly J, Rockenstein E, Mante M, Adame A, Masliah E (2010) Lovastatin ameliorates alpha-synuclein accumulation and oxidation in transgenic mouse models of alpha-synucleinopathies. Exp Neurol 221:267–274.

Korade Z, Kenworthy AK (2008) Lipid rafts, cholesterol, and the brain. Neuropharmacology 55:1265–1273.

Korecka JA, van Kesteren RE, Blaas E, Spitzer SO, Kamstra JH, Smit AB, Swaab DF, Verhaagen J, Bossers K (2013) Phenotypic characterization of retinoic acid differentiated SH-SY5Y cells by transcriptional profiling. PloS One 8:e63862.

Köroğlu Ç, Baysal L, Cetinkaya M, Karasoy H, Tolun A (2013) DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat Disord 19:320–324.

Koshimura K, Tanaka J, Murakami Y, Kato Y (2000) Effects of dopamine and L-DOPA on survival of PC12 cells. J Neurosci Res 62:112–119.

Koudinov AR, Koudinova NV (2003) Cholesterol, synaptic function and Alzheimer's disease. Pharmacopsychiatry 36 Suppl 2:S107–S112.

Krantz DE, Peter D, Liu Y, Edwards RH (1997) Phosphorylation of a vesicular monoamine transporter by casein kinase II. J Biol Chem 272:6752–6759.

Krause MR, Regen SL (2014) The structural role of cholesterol in cell membranes: from condensed bilayers to lipid rafts. Acc Chem Res 47:3512–3521.

Krezel W, Ghyselinck N, Samad TA, Dupé V, Kastner P, Borrelli E, Chambon P (1998) Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science 279:863–867.

Kristensen AS, Andersen J, Jørgensen TN, Sørensen L, Eriksen J, Loland CJ, Strømgaard K, Gether U (2011) SLC6 neurotransmitter transporters: structure, function, and regulation. Pharmacol Rev 63:585–640.

Kumar A, Sharma N, Gupta A, Kalonia H, Mishra J (2012) Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. Brain Res 1471:13–22.

Kurian MA et al. (2011) Clinical and molecular characterisation of hereditary dopamine transporter deficiency syndrome: an observational cohort and experimental study. Lancet Neurol 10:54–62.

Kurosaki R, Muramatsu Y, Watanabe H, Michimata M, Matsubara M, Imai Y, Araki T (2003) Role of dopamine transporter against MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) neurotoxicity in mice. Metab Brain Dis 18:139–146.

Labandeira-Garcia JL, Rodríguez-Perez AI, Villar-Cheda B, Borrajo A, Dominguez-Meijide A, Guerra MJ (2014) Rho Kinase and Dopaminergic Degeneration: A Promising Therapeutic Target for Parkinson's Disease. Neurosci Rev J Bringing Neurobiol Neurol Psychiatry.

Lajtha A, Mikoshiba K (2009) Handbook of neurochemistry and molecular neurobiology neural signaling mechanisms. New York: Springer. Available at: http://site.ebrary.com/id/10305803 [Accessed October 3, 2015].

Lamm O, Ganz J, Melamed E, Offen D (2014) Harnessing neurogenesis for the possible treatment of Parkinson's disease. J Comp Neurol 522:2817–2830.

Lanciego JL, Luquin N, Obeso JA (2012) Functional neuroanatomy of the basal ganglia. Cold Spring Harb Perspect Med 2:a009621.

Lange Y, Ye J, Rigney M, Steck TL (1999) Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. J Lipid Res 40:2264–2270.

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979–980.

Langston JW, Irwin I, Langston EB, Forno LS (1984) 1-Methyl-4-phenylpyridinium ion (MPP+): identification of a metabolite of MPTP, a toxin selective to the substantia nigra. Neurosci Lett 48:87–92.

Lansbury PT, Brice A (2002) Genetics of Parkinson's disease and biochemical studies of implicated gene products. Curr Opin Cell Biol 14:653–660.

Lashuel HA, Petre BM, Wall J, Simon M, Nowak RJ, Walz T, Lansbury PT (2002) Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol 322:1089–1102.

Lee FJ, Liu F, Pristupa ZB, Niznik HB (2001) Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J Off Publ Fed Am Soc Exp Biol 15:916–926.

Lee FJS, Pei L, Liu F (2009) Disruption of the dopamine transporter-dopamine D2 receptor interaction in schizophrenia. Synap N Y N 63:710–712.

Leegwater-Kim J, Waters C (2007) Role of tolcapone in the treatment of Parkinson's disease. Expert Rev Neurother 7:1649–1657.

Lee H-J, Khoshaghideh F, Lee S, Lee S-J (2006) Impairment of microtubule-dependent trafficking by overexpression of alpha-synuclein. Eur J Neurosci 24:3153–3162.

Lee K-H, Kim M-Y, Kim D-H, Lee Y-S (2004) Syntaxin 1A and receptor for activated C kinase interact with the N-terminal region of human dopamine transporter. Neurochem Res 29:1405–1409.

Lee PC, Bordelon Y, Bronstein J, Sinsheimer JS, Farrer M, Ritz B (2015) Head injury, α-synuclein genetic variability and Parkinson's disease. Eur J Neurol Off J Eur Fed Neurol Soc 22:874–878.

Lee SJ, Sekimoto T, Yamashita E, Nagoshi E, Nakagawa A, Imamoto N, Yoshimura M, Sakai H, Chong KT, Tsukihara T, Yoneda Y (2003) The structure of importin-beta bound to SREBP-2: nuclear import of a transcription factor. Science 302:1571–1575.

Lee YC, Lin CH, Wu RM, Lin MS, Lin JW, Chang CH, Lai MS (2013) Discontinuation of statin therapy associates with Parkinson disease: a population-based study. Neurology 81:410–416.

Lehmensiek V, Tan E-M, Liebau S, Lenk T, Zettlmeisl H, Schwarz J, Storch A (2006) Dopamine transporter-mediated cytotoxicity of 6-hydroxydopamine in vitro depends on expression of mutant alpha-synucleins related to Parkinson's disease. Neurochem Int 48:329–340.

Lehmensiek V, Tan E-M, Schwarz J, Storch A (2002) Expression of mutant alpha-synucleins enhances dopamine transporter-mediated MPP+ toxicity in vitro. Neuroreport 13:1279–1283.

Leoni V, Caccia C (2013) 24S-hydroxycholesterol in plasma: a marker of cholesterol turnover in neurodegenerative diseases. Biochimie 95:595–612.

Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH (1998) The ubiquitin pathway in Parkinson's disease. Nature 395:451–452.

Lestavel S, Fruchart JC (1994) Lipoprotein receptors. Cell Mol Biol Noisy--Gd Fr 40:461–481.

Leverenz JB, Umar I, Wang Q, Montine TJ, McMillan PJ, Tsuang DW, Jin J, Pan C, Shin J, Zhu D, Zhang J (2007) Proteomic identification of novel proteins in cortical lewy bodies. Brain Pathol Zurich Switz 17:139–145.

Leviel V (2011) Dopamine release mediated by the dopamine transporter, facts and consequences. J Neurochem 118:475–489.

Le W, Conneely OM, He Y, Jankovic J, Appel SH (1999) Reduced Nurr1 expression increases the vulnerability of mesencephalic dopamine neurons to MPTP-induced injury. J Neurochem 73:2218–2221.

LeWitt PA et al. (2011) AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. Lancet Neurol 10:309–319.

Lewy F (1912) Paralysis agitans. in Pathologische Anatomie., ed Lewandowsky M. Berlin: Julius Springer-Verlag.

Le W, Zhang L, Xie W, Li S, Dani JA (2015) Pitx3 deficiency produces decreased dopamine signaling and induces motor deficits in Pitx3(-/-) mice. Neurobiol Aging.

Liao JK, Laufs U (2005) Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 45:89–118.

Lieberman AN, Goldstein M, Gopinathan G, Neophytides A, Hiesiger E, Walker R, Nelson J (1984) Combined use of benserazide and carbidopa in Parkinson's disease. Neurology 34:227–229.

Li J-Q, Tan L, Yu J-T (2014) The role of the LRRK2 gene in Parkinsonism. Mol Neurodegener 9:47.

Li L-B, Chen N, Ramamoorthy S, Chi L, Cui X-N, Wang LC, Reith MEA (2004) The role of N-glycosylation in function and surface trafficking of the human dopamine transporter. J Biol Chem 279:21012–21020.

Li L-H, Qin H-Z, Wang J-L, Wang J, Wang X-L, Gao G-D (2009a) Axonal degeneration of nigra-striatum dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. J Int Med Res 37:455–463.

Li L, Zhang W, Cheng S, Cao D, Parent M (2012) Isoprenoids and related pharmacological interventions: potential application in Alzheimer's disease. Mol Neurobiol 46:64–77.

Lim K-L, Tan JMM (2007) Role of the ubiquitin proteasome system in Parkinson's disease. BMC Biochem 8 Suppl 1:S13.

Limousin P, Pollak P, Benazzouz A, Hoffmann D, Le Bas JF, Broussolle E, Perret JE, Benabid AL (1995) Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. Lancet Lond Engl 345:91–95.

Lin C-H, Tsai P-I, Wu R-M, Chien C-T (2010a) LRRK2 G2019S mutation induces dendrite degeneration through mislocalization and phosphorylation of tau by recruiting autoactivated GSK3ß. J Neurosci Off J Soc Neurosci 30:13138–13149.

Lingor P, Koch JC, Tönges L, Bähr M (2012) Axonal degeneration as a therapeutic target in the CNS. Cell Tissue Res 349:289–311.

Lin Z, Zhang P-W, Zhu X, Melgari J-M, Huff R, Spieldoch RL, Uhl GR (2003) Phosphatidylinositol 3-kinase, protein kinase C, and MEK1/2 kinase regulation of dopamine transporters (DAT) require N-terminal DAT phosphoacceptor sites. J Biol Chem 278:20162–20170.

Lin Z, Zhao Y, Chung CY, Zhou Y, Xiong N, Glatt CE, Isacson O (2010b) High regulatability favors genetic selection in SLC18A2, a vesicular monoamine transporter essential for life. FASEB J Off Publ Fed Am Soc Exp Biol 24:2191–2200.

Liscum L, Ruggiero RM, Faust JR (1989) The intracellular transport of low density lipoprotein-derived cholesterol is defective in Niemann-Pick type C fibroblasts. J Cell Biol 108:1625–1636.

Liu Y, Edwards RH (1997) The role of vesicular transport proteins in synaptic transmission and neural degeneration. Annu Rev Neurosci 20:125–156.

Liu Y, Schweitzer ES, Nirenberg MJ, Pickel VM, Evans CJ, Edwards RH (1994) Preferential localization of a vesicular monoamine transporter to dense core vesicles in PC12 cells. J Cell Biol 127:1419–1433.

Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C (2009b) Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci 12:826–828.

Li Z, Dong X, Dong X, Wang Z, Liu W, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L, Wu D (2005) Regulation of PTEN by Rho small GTPases. Nat Cell Biol 7:399–404.

Lohr KM, Bernstein AI, Stout KA, Dunn AR, Lazo CR, Alter SP, Wang M, Li Y, Fan X, Hess EJ, Yi H, Vecchio LM, Goldstein DS, Guillot TS, Salahpour A, Miller GW (2014) Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. Proc Natl Acad Sci U A 111:9977–9982.

Lohr KM, Miller GW (2014) VMAT2 and Parkinson's disease: harnessing the dopamine vesicle. Expert Rev Neurother 14:1115–1117.

Lohr KM, Stout KA, Dunn AR, Wang M, Salahpour A, Guillot TS, Miller GW (2015) Increased Vesicular Monoamine Transporter 2 (VMAT2; Slc18a2) Protects against Methamphetamine Toxicity. ACS Chem Neurosci 6:790–799.

Lopes FM, Schroder R, da Frota ML, Zanotto-Filho A, Muller CB, Pires AS, Meurer RT, Colpo GD, Gelain DP, Kapczinski F, Moreira JC, Fernandes Mda C, Klamt F (2010) Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res 1337:85–94.

Lotharius J, Brundin P (2002) Pathogenesis of parkinson's disease: dopamine, vesicles and α-synuclein. Nat Rev Neurosci 3:932–942.

Lowery LA, Van Vactor D (2009) The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 10:332–343.

Lu D, Qu C, Goussev A, Jiang H, Lu C, Schallert T, Mahmood A, Chen J, Li Y, Chopp M (2007) Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J Neurotrauma 24:1132–1146.

Luk KC, Rymar VV, van den Munckhof P, Nicolau S, Steriade C, Bifsha P, Drouin J, Sadikot AF (2013) The transcription factor Pitx3 is expressed selectively in midbrain dopaminergic neurons susceptible to neurodegenerative stress. J Neurochem 125:932–943.

Lu X, Kim-Han JS, Harmon S, Sakiyama-Elbert SE, O'Malley KL (2014) The Parkinsonian mimetic, 6-OHDA, impairs axonal transport in dopaminergic axons. Mol Neurodegener 9:17.

Lv J-H, He L, Sui S-F (2008) Lipid rafts association of synaptotagmin I on synaptic vesicles. Biochem Biokhimiia 73:283–288.

MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A (2006) The familial Parkinsonism gene LRRK2 regulates neurite process morphology. Neuron 52:587–593.

Maden M (2007) Retinoic acid in the development, regeneration and maintenance of the nervous system. Nat Rev Neurosci 8:755–765.

Maguire-Zeiss KA, Short DW, Federoff HJ (2005) Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Brain Res Mol Brain Res 134:18–23.

Malfitano AM, Marasco G, Proto MC, Laezza C, Gazzerro P, Bifulco M (2014) Statins in neurological disorders: an overview and update. Pharmacol Res 88:74–83.

Mandel S, Grünblatt E, Riederer P, Gerlach M, Levites Y, Youdim MBH (2003) Neuroprotective strategies in Parkinson's disease : an update on progress. CNS Drugs 17:729–762.

Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129:1261–1274.

Manzoni C, Lewis PA (2013) Dysfunction of the autophagy/lysosomal degradation pathway is a shared feature of the genetic synucleinopathies. FASEB J Off Publ Fed Am Soc Exp Biol 27:3424–3429.

Ma PT, Gil G, Südhof TC, Bilheimer DW, Goldstein JL, Brown MS (1986) Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. Proc Natl Acad Sci U S A 83:8370–8374.

Marconi S, Zwingers T (2014) Comparative efficacy of selegiline versus rasagiline in the treatment of early Parkinson's disease. Eur Rev Med Pharmacol Sci 18:1879–1882.

Mark M, Muller P, Maier R, Eisele B (1996) Effects of a novel 2,3-oxidosqualene cyclase inhibitor on the regulation of cholesterol biosynthesis in HepG2 cells. J Lipid Res 37:148–158.

Marsala SZ, Gioulis M, Ceravolo R, Tinazzi M (2012) A systematic review of catechol-0-methyltransferase inhibitors: efficacy and safety in clinical practice. Clin Neuropharmacol 35:185–190.

Marshall V, Grosset D (2003) Role of dopamine transporter imaging in routine clinical practice. Mov Disord Off J Mov Disord Soc 18:1415–1423.

Martinez A ed. (2014) Emerging drugs and targets for Parkinson's disease. Cambridge: RSC.

Martins JB, Bastos M de L, Carvalho F, Capela JP (2013) Differential Effects of Methyl-4-Phenylpyridinium Ion, Rotenone, and Paraquat on Differentiated SH-SY5Y Cells. J Toxicol 2013:347312.

Martin WRW, Wieler M, Stoessl AJ, Schulzer M (2008) Dihydrotetrabenazine positron emission tomography imaging in early, untreated Parkinson's disease. Ann Neurol 63:388–394.

Marxreiter F, Regensburger M, Winkler J (2013) Adult neurogenesis in Parkinson's disease. Cell Mol Life Sci CMLS 70:459–473.

Masoud ST, Vecchio LM, Bergeron Y, Hossain MM, Nguyen LT, Bermejo MK, Kile B, Sotnikova TD, Siesser WB, Gainetdinov RR, Wightman RM, Caron MG, Richardson JR, Miller GW, Ramsey AJ, Cyr M, Salahpour A (2015) Increased expression of the dopamine transporter leads to loss of dopamine neurons, oxidative stress and l-DOPA reversible motor deficits. Neurobiol Dis 74:66–75.

Massano J, Bhatia KP (2012) Clinical approach to Parkinson's disease: features, diagnosis, and principles of management. Cold Spring Harb Perspect Med 2:a008870.

Masserano JM, Gong L, Kulaga H, Baker I, Wyatt RJ (1996) Dopamine induces apoptotic cell death of a catecholaminergic cell line derived from the central nervous system. Mol Pharmacol 50:1309–1315.

Massy ZA, Keane WF, Kasiske BL (1996) Inhibition of the mevalonate pathway: benefits beyond cholesterol reduction? Lancet Lond Engl 347:102–103.

Masuda-Suzukake M, Nonaka T, Hosokawa M, Oikawa T, Arai T, Akiyama H, Mann DMA, Hasegawa M (2013) Prion-like spreading of pathological α-synuclein in brain. Brain J Neurol 136:1128–1138.

Matsuda S, Nakanishi A, Wada Y, Kitagishi Y (2013) Roles of PI3K/AKT/PTEN Pathway as a Target for Pharmaceutical Therapy. Open Med Chem J 7:23–29.

McCaffery P, Dräger UC (1994) High levels of a retinoic acid-generating dehydrogenase in the meso-telencephalic dopamine system. Proc Natl Acad Sci U S A 91:7772–7776.

McFarland AJ, Anoopkumar-Dukie S, Arora DS, Grant GD, McDermott CM, Perkins AV, Davey AK (2014) Molecular mechanisms underlying the effects of statins in the central nervous system. Int J Mol Sci 15:20607–20637.

McFarland K, Spalding TA, Hubbard D, Ma J-N, Olsson R, Burstein ES (2013) Low dose bexarotene treatment rescues dopamine neurons and restores behavioral function in models of Parkinson's disease. ACS Chem Neurosci 4:1430–1438.

McGeer PL, Itagaki S, Akiyama H, McGeer EG (1988) Rate of cell death in parkinsonism indicates active neuropathological process. Ann Neurol 24:574–576.

McGuinness B, Craig D, Bullock R, Malouf R, Passmore P (2014) Statins for the treatment of dementia. Cochrane Database Syst Rev 7:CD007514.

McTaggart SJ (2006) Isoprenylated proteins. Cell Mol Life Sci CMLS 63:255–267.

Meador-Woodruff JH, Mansour A (1991) A. E. Bennett Award paper. Expression of the dopamine D2 receptor gene in brain. Biol Psychiatry 30:985–1007.

Meireles J, Massano J (2012) Cognitive impairment and dementia in Parkinson's disease: clinical features, diagnosis, and management. Front Neurol 3:88.

Meiser J, Weindl D, Hiller K (2013) Complexity of dopamine metabolism. Cell Commun Signal CCS 11:34.

Meissner W, Prunier C, Guilloteau D, Chalon S, Gross CE, Bezard E (2003) Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. Mol Neurobiol 28:209–218.

Melikian HE, Buckley KM (1999) Membrane trafficking regulates the activity of the human dopamine transporter. J Neurosci Off J Soc Neurosci 19:7699–7710.

Meynert TH, Sachs B, Jackson S (1968) Psychiatry, a clinical treatise on diseases of the fore-brain based upon a study of its structure, functions and nutrition; Part I. (all published) The anatomy, physiology, and chemistry of the brain. A facsimile of the 1885 edition with a new introduction,. New York: Hafner.

Mihos CG, Pineda AM, Santana O (2014) Cardiovascular effects of statins, beyond lipid-lowering properties. Pharmacol Res 88:12–19.

Miller GW, Gainetdinov RR, Levey AI, Caron MG (1999) Dopamine transporters and neuronal injury. Trends Pharmacol Sci 20:424–429.

Miller GW, Wang YM, Gainetdinov RR, Caron MG (2001) Dopamine transporter and vesicular monoamine transporter knockout mice : implications for Parkinson's disease. Methods Mol Med 62:179–190.

Miranda M, Dionne KR, Sorkina T, Sorkin A (2007) Three ubiquitin conjugation sites in the amino terminus of the dopamine transporter mediate protein kinase C-dependent endocytosis of the transporter. Mol Biol Cell 18:313–323.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors: from structure to function. Physiol Rev 78:189–225.

Miyano K, Sumimoto H (2012) Assessment of the role for Rho family GTPases in NADPH oxidase activation. Methods Mol Biol Clifton NJ 827:195–212.

Miyazaki I, Asanuma M (2008) Dopaminergic neuron-specific oxidative stress caused by dopamine itself. Acta Med Okayama 62:141–150.

Miziorko HM (2011) Enzymes of the mevalonate pathway of isoprenoid biosynthesis. Arch Biochem Biophys 505:131–143.

Moldovan A-S, Groiss SJ, Elben S, Südmeyer M, Schnitzler A, Wojtecki L (2015) The treatment of Parkinson's disease with deep brain stimulation: current issues. Neural Regen Res 10:1018–1022.

Morales I, Sanchez A, Rodriguez-Sabate C, Rodriguez M (2015) The degeneration of dopaminergic synapses in Parkinson's disease: A selective animal model. Behav Brain Res 289:19–28.

Morciano M, Burre J, Corvey C, Karas M, Zimmermann H, Volknandt W (2005) Immunoisolation of two synaptic vesicle pools from synaptosomes: a proteomics analysis. J Neurochem 95:1732–1745.

Moreno-Manzano V, Ishikawa Y, Lucio-Cazana J, Kitamura M (1999) Suppression of apoptosis by all-trans-retinoic acid. Dual intervention in the c-Jun n-terminal kinase-AP-1 pathway. J Biol Chem 274:20251–20258.

Morfini G, Pigino G, Opalach K, Serulle Y, Moreira JE, Sugimori M, Llinás RR, Brady ST (2007) 1-Methyl-4-phenylpyridinium affects fast axonal transport by activation of caspase and protein kinase C. Proc Natl Acad Sci U S A 104:2442–2447.

Morgan JC, Currie LJ, Harrison MB, Bennett JP, Trugman JM, Wooten GF (2014) Mortality in levodopa-treated Parkinson's disease. Park Dis 2014:426976.

Mortensen OV, Amara SG (2003) Dynamic regulation of the dopamine transporter. Eur J Pharmacol 479:159–170.

Mosharov EV, Larsen KE, Kanter E, Phillips KA, Wilson K, Schmitz Y, Krantz DE, Kobayashi K, Edwards RH, Sulzer D (2009) Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. Neuron 62:218–229.

Mosharov EV, Staal RGW, Bové J, Prou D, Hananiya A, Markov D, Poulsen N, Larsen KE, Moore CMH, Troyer MD, Edwards RH, Przedborski S, Sulzer D (2006) Alpha-synuclein overexpression increases cytosolic catecholamine concentration. J Neurosci Off J Soc Neurosci 26:9304–9311.

Moszczynska A, Saleh J, Zhang H, Vukusic B, Lee FJS, Liu F (2007) Parkin disrupts the alpha-synuclein/dopamine transporter interaction: consequences toward dopamine-induced toxicity. J Mol Neurosci MN 32:217–227.

Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease (2003) The Unified Parkinson's Disease Rating Scale (UPDRS): status and recommendations. Mov Disord Off J Mov Disord Soc 18:738–750.

Müller MLTM, Bohnen NI (2013) Cholinergic dysfunction in Parkinson's disease. Curr Neurol Neurosci Rep 13:377.

Muñoz P, Paris I, Sanders LH, Greenamyre JT, Segura-Aguilar J (2012) Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. Biochim Biophys Acta 1822:1125–1136.

Musacchio JM, D'Angelo GL, McQueen CA (1971) Dihydropteridine reductase: implication on the regulation of catecholamine biosynthesis. Proc Natl Acad Sci U S A 68:2087–2091.

Myant NB (1982) Cholesterol transport through the plasma. Clin Sci Lond Engl 1979 62:261–271.

Myöhänen TT, Schendzielorz N, Männistö PT (2010) Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. J Neurochem 113:1632–1643.

Nagatsua T, Sawadab M (2009) L-dopa therapy for Parkinson's disease: past, present, and future. Parkinsonism Relat Disord 15 Suppl 1:S3–S8.

Nagatsu T (1995) Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. Essays Biochem 30:15–35.

Nagatsu T, Levitt M, Udenfriend S (1964) TYROSINE HYDROXYLASE. THE INITIAL STEP IN NOREPINEPHRINE BIOSYNTHESIS. J Biol Chem 239:2910–2917.

Nakashima Y, Sanada H, Suzue R (1971) Induction of tyrosine hydroxylase by dibutyryl adenosine 3',5'-monophosphate in guinea pig adrenal slices. Arch Biochem Biophys 145:354–357.

Nandhagopal R, Kuramoto L, Schulzer M, Mak E, Cragg J, McKenzie J, McCormick S, Ruth TJ, Sossi V, de la Fuente-Fernandez R, Stoessl AJ (2011) Longitudinal evolution of compensatory changes in striatal dopamine processing in Parkinson's disease. Brain J Neurol 134:3290–3298.

Napolitano A, Cesura AM, Da Prada M (1995) The role of monoamine oxidase and catechol O-methyltransferase in dopaminergic neurotransmission. J Neural Transm Suppl 45:35–45.

Needham D, Nunn RS (1990) Elastic deformation and failure of lipid bilayer membranes containing cholesterol. Biophys J 58:997–1009.

Neher E, Sakaba T (2008) Multiple roles of calcium ions in the regulation of neurotransmitter release. Neuron 59:861–872.

Nes WD (2011) Biosynthesis of cholesterol and other sterols. Chem Rev 111:6423–6451.

Nguyen ML, Cox GD, Parsons SM (1998) Kinetic parameters for the vesicular acetylcholine transporter: two protons are exchanged for one acetylcholine. Biochemistry (Mosc) 37:13400–13410.

Nirenberg MJ, Chan J, Liu Y, Edwards RH, Pickel VM (1996) Ultrastructural localization of the vesicular monoamine transporter-2 in midbrain dopaminergic neurons: potential sites for somatodendritic storage and release of dopamine. J Neurosci 16:4135–4145.

Nirenberg MJ, Chan J, Pohorille A, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1997a) The dopamine transporter: comparative ultrastructure of dopaminergic axons in limbic and motor compartments of the nucleus accumbens. J Neurosci 17:6899–6907.

Nirenberg MJ, Chan J, Pohorille A, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1997b) The dopamine transporter: comparative ultrastructure of dopaminergic axons in limbic and motor compartments of the nucleus accumbens. J Neurosci 17:6899–6907.

Nirenberg MJ, Chan J, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1997c) Immunogold localization of the dopamine transporter: an ultrastructural study of the rat ventral tegmental area. J Neurosci Off J Soc Neurosci 17:5255–5262.

Nirenberg MJ, Liu Y, Peter D, Edwards RH, Pickel VM (1995) The vesicular monoamine transporter 2 is present in small synaptic vesicles and preferentially localizes to large dense core vesicles in rat solitary tract nuclei. Proc Natl Acad Sci 92:8773–8777.

Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP (2003) Pitx3 is required for development of substantia nigra dopaminergic neurons. Proc Natl Acad Sci U S A 100:4245–4250.

Nutt JG, Carter JH, Sexton GJ (2004) The dopamine transporter: importance in Parkinson's disease. Ann Neurol 55:766–773.

Obeso JA, Marin C, Rodriguez-Oroz C, Blesa J, Benitez-Temiño B, Mena-Segovia J, Rodríguez M, Olanow CW (2008) The basal ganglia in Parkinson's disease: current concepts and unexplained observations. Ann Neurol 64 Suppl 2:S30–S46.

O'Connor DM, Boulis NM (2015) Gene therapy for neurodegenerative diseases. Trends Mol Med 21:504–512.

Oh S-M, Chang M-Y, Song J-J, Rhee Y-H, Joe E-H, Lee H-S, Yi S-H, Lee S-H (2015) Combined Nurr1 and Foxa2 roles in the therapy of Parkinson's disease. EMBO Mol Med 7:510–525.

Olgiati S, De Rosa A, Quadri M, Criscuolo C, Breedveld GJ, Picillo M, Pappatà S, Quarantelli M, Barone P, De Michele G, Bonifati V (2014) PARK20 caused by SYNJ1 homozygous Arg258Gln mutation in a new Italian family. Neurogenetics 15:183–188.

Olmstead AD, Knecht W, Lazarov I, Dixit SB, Jean F (2012) Human subtilase SKI-1/S1P is a master regulator of the HCV Lifecycle and a potential host cell target for developing indirect-acting antiviral agents. PLoS Pathog 8:e1002468.

O'Malley KL (2010) The role of axonopathy in Parkinson's disease. Exp Neurobiol 19:115–119.

Ondo WG, Tintner R, Thomas M, Jankovic J (2002) Tetrabenazine treatment for Huntington's disease-associated chorea. Clin Neuropharmacol 25:300–302.

Orth M, Bellosta S (2012) Cholesterol: its regulation and role in central nervous system disorders. Cholesterol 2012:292598.

Oster GF, Cheng LY, Moore HP, Perelson AS (1989) Vesicle formation in the Golgi apparatus. J Theor Biol 141:463–504.

Pagonabarraga J, Kulisevsky J (2012) Cognitive impairment and dementia in Parkinson's disease. Neurobiol Dis 46:590–596.

Pahan K, Liu X, McKinney MJ, Wood C, Sheikh FG, Raymond JR (2000) Expression of a dominant-negative mutant of p21(ras) inhibits induction of nitric oxide synthase and activation of nuclear factor-kappaB in primary astrocytes. J Neurochem 74:2288–2295.

Pahan K, Sheikh FG, Khan M, Namboodiri AM, Singh I (1998) Sphingomyelinase and ceramide stimulate the expression of inducible nitric-oxide synthase in rat primary astrocytes. J Biol Chem 273:2591–2600.

Pahan K, Sheikh FG, Namboodiri AM, Singh I (1997) Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. J Clin Invest 100:2671–2679.

Pahapill PA, Lozano AM (2000) The pedunculopontine nucleus and Parkinson's disease. Brain J Neurol 123 ( Pt 9):1767–1783.

Paisán-Ruíz C et al. (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44:595–600.

Palfi S et al. (2014) Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. Lancet Lond Engl 383:1138–1146.

Palinski W, Napoli C (2002) Unraveling pleiotropic effects of statins on plaque rupture. Arterioscler Thromb Vasc Biol 22:1745–1750.

Palsuledesai CC, Distefano MD (2015) Protein prenylation: enzymes, therapeutics, and biotechnology applications. ACS Chem Biol 10:51–62.

Pan T, Xie W, Jankovic J, Le W (2005) Biological effects of pramipexole on dopaminergic neuron-associated genes: relevance to neuroprotection. Neurosci Lett 377:106–109.

Pápai P, Pax corporis (1690) az az az emberi testnek belső nyavalyáinak okairól, fészkeiről 's azoknak orvoslásának módgyáról való tracta …, ("Pax corporis, i.e. a teaching of the causes, sources and the methods of treatment of the internal diseases of the human body"). Kolozsvár: Némethi Mihály.

Parisiadou L, Cai H (2010) LRRK2 function on actin and microtubule dynamics in Parkinson disease. Commun Integr Biol 3:396–400.

Parisiadou L, Xie C, Cho HJ, Lin X, Gu X-L, Long C-X, Lobbestael E, Baekelandt V, Taymans J-M, Sun L, Cai H (2009) Phosphorylation of ezrin/radixin/moesin proteins by LRRK2 promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. J Neurosci Off J Soc Neurosci 29:13971–13980.

Park C-H, Kang JS, Shin YH, Chang M-Y, Chung S, Koh H-C, Zhu MH, Oh SB, Lee Y-S, Panagiotakos G, Tabar V, Studer L, Lee S-H (2006) Acquisition of in vitro and in vivo functionality of Nurr1-induced dopamine neurons. FASEB J Off Publ Fed Am Soc Exp Biol 20:2553–2555.

Parkinson J (1817) An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci 14:223–236; discussion 222.

Park J, Matralis AN, Berghuis AM, Tsantrizos YS (2014) Human isoprenoid synthase enzymes as therapeutic targets. Front Chem 2:50.

Parti R, Ozkan ED, Harnadek GJ, Njus D (1987) Inhibition of norepinephrine transport and reserpine binding by reserpine derivatives. J Neurochem 48:949–953.

Patel A, Uhl G, Kuhar MJ (1993) Species differences in dopamine transporters: postmortem changes and glycosylation differences. J Neurochem 61:496–500.

Paul R, Choudhury A, Borah A (2015) Cholesterol - A putative endogenous contributor towards Parkinson's disease. Neurochem Int.

Paumier KL, Siderowf AD, Auinger P, Oakes D, Madhavan L, Espay AJ, Revilla FJ, Collier TJ, Parkinson Study Group Genetics Epidemiology Working Group (2012) Tricyclic antidepressants delay the need for dopaminergic therapy in early Parkinson's disease. Mov Disord Off J Mov Disord Soc 27:880–887.

Paus M, Kohl Z, Ben Abdallah NM-B, Galter D, Gillardon F, Winkler J (2013) Enhanced dendritogenesis and axogenesis in hippocampal neuroblasts of LRRK2 knockout mice. Brain Res 1497:85–100.

Payne AH, Hales DB (2004) Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev 25:947–970.

Perlmutter JS (2009) Assessment of Parkinson disease manifestations. Curr Protoc Neurosci Editor Board Jacqueline N Crawley Al Chapter 10:Unit10.1.

Perlmutter JS, Mink JW (2006) Deep brain stimulation. Annu Rev Neurosci 29:229–257.

Peter D, Liu Y, Sternini C, de Giorgio R, Brecha N, Edwards RH (1995) Differential expression of two vesicular monoamine transporters. J Neurosci Off J Soc Neurosci 15:6179–6188.

Petrinovic MM, Duncan CS, Bourikas D, Weinman O, Montani L, Schroeter A, Maerki D, Sommer L, Stoeckli ET, Schwab ME (2010) Neuronal Nogo-A regulates neurite fasciculation, branching and extension in the developing nervous system. Dev Camb Engl 137:2539–2550.

Pfrieger FW (2003) Role of cholesterol in synapse formation and function. Biochim Biophys Acta 1610:271–280.

Pfrieger FW, Ungerer N (2011) Cholesterol metabolism in neurons and astrocytes. Prog Lipid Res 50:357–371.

Pham CLL, Leong SL, Ali FE, Kenche VB, Hill AF, Gras SL, Barnham KJ, Cappai R (2009) Dopamine and the dopamine oxidation product 5,6-dihydroxylindole promote distinct on-pathway and off-pathway aggregation of alpha-synuclein in a pH-dependent manner. J Mol Biol 387:771–785.

Phillips JE, Johnson WJ (1998) Efflux and Plasma Transport of Biosynthetic Sterols. In: Intracellular Cholesterol Trafficking (Chang TY, Freeman DA, eds), pp 147–168. Boston, MA: Springer US. Available at: http://link.springer.com/10.1007/978-1-4615-5113-3_10 [Accessed September 17, 2015].

Phillips JH, Apps DK (1980) Stoichiometry of catecholamine/proton exchange across the chromaffin-granule membrane. Biochem J 192:273–278.

Pifl C, Giros B, Caron MG (1993) Dopamine transporter expression confers cytotoxicity to low doses of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium. J Neurosci Off J Soc Neurosci 13:4246–4253.

Pifl C, Rajput A, Reither H, Blesa J, Cavada C, Obeso JA, Rajput AH, Hornykiewicz O (2014) Is Parkinson's Disease a Vesicular Dopamine Storage Disorder? Evidence from a Study in Isolated Synaptic Vesicles of Human and Nonhuman Primate Striatum. J Neurosci 34:8210–8218.

Pike LJ (2009) The challenge of lipid rafts. J Lipid Res 50 Suppl:S323–S328.

Pinder RM, Brogden RN, Sawyer PR, Speight TM, Avery GS (1976) Levodopa and decarboxylase inhibitors: a review of their clinical pharmacology and use in the treatment of parkinsonism. Drugs 11:329–377.

Plowey ED, Cherra SJ, Liu Y-J, Chu CT (2008) Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. J Neurochem 105:1048–1056.

Plum LA, Clagett-Dame M (1996) All-trans retinoic acid stimulates and maintains neurite outgrowth in nerve growth factor-supported developing chick embryonic sympathetic neurons. Dev Dyn Off Publ Am Assoc Anat 205:52–63.

Politis M (2014) Neuroimaging in Parkinson disease: from research setting to clinical practice. Nat Rev Neurol 10:708–722.

Politis M, Loane C (2011) Serotonergic dysfunction in Parkinson's disease and its relevance to disability. ScientificWorldJournal 11:1726–1734.

Pollak P, Benabid AL, Gross C, Gao DM, Laurent A, Benazzouz A, Hoffmann D, Gentil M, Perret J (1993) [Effects of the stimulation of the subthalamic nucleus in Parkinson disease]. Rev Neurol (Paris) 149:175–176.

Polymeropoulos MH et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047.

Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, Stenroos ES, Pho LT, Schaffer AA, Lazzarini AM, Nussbaum RL, Duvoisin RC (1996) Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. Science 274:1197–1199.

Pooler AM, Xi SC, Wurtman RJ (2006) The 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitor pravastatin enhances neurite outgrowth in hippocampal neurons. J Neurochem 97:716–723.

Popjak G (1958) Biosynthesis of cholesterol and related substances. Annu Rev Biochem 27:533–560.

Porter JA, Young KE, Beachy PA (1996) Cholesterol modification of hedgehog signaling proteins in animal development. Science 274:255–259.

Prasad V (2014) Statins, primary prevention, and overall mortality. Ann Intern Med 160:867–869.

Presgraves SP, Ahmed T, Borwege S, Joyce JN (2004) Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. Neurotox Res 5:579–598.

Profumo E, Buttari B, Saso L, Rigano R (2014) Pleiotropic effects of statins in atherosclerotic disease: focus on the antioxidant activity of atorvastatin. Curr Top Med Chem 14:2542–2551.

Prots I, Veber V, Brey S, Campioni S, Buder K, Riek R, Böhm KJ, Winner B (2013) α-Synuclein oligomers impair neuronal microtubule-kinesin interplay. J Biol Chem 288:21742–21754.

Przedborski S, Levivier M, Jiang H, Ferreira M, Jackson-Lewis V, Donaldson D, Togasaki DM (1995) Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. Neuroscience 67:631–647.

Puttagunta R, Di Giovanni S (2011) Retinoic acid signaling in axonal regeneration. Front Mol Neurosci 4:59.

Qin ZH, Chen JF, Weiss B (1994) Lesions of mouse striatum induced by 6-hydroxydopamine differentially alter the density, rate of synthesis, and level of gene expression of D1 and D2 dopamine receptors. J Neurochem 62:411–420.

Qi Z, Miller GW, Voit EO (2008) Computational systems analysis of dopamine metabolism. PloS One 3:e2444.

Quik M, Perez XA, Bordia T (2012) Nicotine as a potential neuroprotective agent for Parkinson's disease. Mov Disord Off J Mov Disord Soc 27:947–957.

Quinn NP (1997) Parkinson's disease: clinical features. Baillières Clin Neurol 6:1–13.

Rabinovic AD, Lewis DA, Hastings TG (2000) Role of oxidative changes in the degeneration of dopamine terminals after injection of neurotoxic levels of dopamine. Neuroscience 101:67–76.

Racchetti G, Lorusso A, Schulte C, Gavello D, Carabelli V, D'Alessandro R, Meldolesi J (2010) Rapid neurite outgrowth in neurosecretory cells and neurons is sustained by the exocytosis of a cytoplasmic organelle, the enlargeosome. J Cell Sci 123:165–170.

Raina V, Gupta S, Yadav S, Surolia A (2013) Simvastatin induced neurite outgrowth unveils role of cell surface cholesterol and acetyl CoA carboxylase in SH-SY5Y cells. PLoS One 8:e74547.

Rainbow TC, Parsons B, Wieczorek CM, Manaker S (1985) Localization in rat brain of binding sites for parkinsonian toxin MPTP: similarities with [3H]pargyline binding to monoamine oxidase. Brain Res 330:337–342.

Rajput AH, Rajput A, Lang AE, Kumar R, Uitti RJ, Galvez-Jimenez N (1998) New use for an old drug: amantadine benefits levodopa-induced dyskinesia. Mov Disord Off J Mov Disord Soc 13:851.

Ramanan VK, Saykin AJ (2013) Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. Am J Neurodegener Dis 2:145–175.

Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat A-L, Roeper J, Al-Din A, Hillmer AM, Karsak M, Liss B, Woods CG, Behrens MI, Kubisch C (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 38:1184–1191.

Ramirez C, Tercero I, Pineda A, Burgos JS (2011) Simvastatin is the statin that most efficiently protects against kainate-induced excitotoxicity and memory impairment. J Alzheimers Dis JAD 24:161–174.

Ramonet D et al. (2011) Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. PloS One 6:e18568.

Ramsay RR, Singer TP (1986) Energy-dependent uptake of N-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria. J Biol Chem 261:7585–7587.

Rappold PM, Cui M, Chesser AS, Tibbett J, Grima JC, Duan L, Sen N, Javitch JA, Tieu K (2011) Paraquat neurotoxicity is mediated by the dopamine transporter and organic cation transporter-3. Proc Natl Acad Sci 108:20766–20771.

Rappold PM, Tieu K (2010) Astrocytes and therapeutics for Parkinson's disease. Neurother J Am Soc Exp Neurother 7:413–423.

Reiss AB, Wirkowski E (2007) Role of HMG-CoA reductase inhibitors in neurological disorders : progress to date. Drugs 67:2111–2120.

Reiss AB, Wirkowski E (2009) Statins in neurological disorders: mechanisms and therapeutic value. ScientificWorldJournal 9:1242–1259.

Ren Y, Liu W, Jiang H, Jiang Q, Feng J (2005) Selective vulnerability of dopaminergic neurons to microtubule depolymerization. J Biol Chem 280:34105–34112.

Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev 14:2819–2830.

Rhinn M, Dollé P (2012) Retinoic acid signalling during development. Dev Camb Engl 139:843–858.

Riachi NJ, LaManna JC, Harik SI (1989) Entry of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine into the rat brain. J Pharmacol Exp Ther 249:744–748.

Rilstone JJ, Alkhater RA, Minassian BA (2013) Brain dopamine-serotonin vesicular transport disease and its treatment. N Engl J Med 368:543–550.

Rise P, Ghezzi S, Carissimi R, Mastromauro F, Petroni A, Galli C (2007) Delta5 desaturase mRNA levels are increased by simvastatin via SREBP-1 at early stages, not via PPARalpha, in THP-1 cells. Eur J Pharmacol 571:97–105.

Risselada HJ, Grubmüller H (2012) How SNARE molecules mediate membrane fusion: recent insights from molecular simulations. Curr Opin Struct Biol 22:187–196.

Rizzolatti G, Luppino G (2001) The cortical motor system. Neuron 31:889–901.

Rizzoli SO (2014) Synaptic vesicle recycling: steps and principles. EMBO J 33:788–822.

Rizzoli SO, Betz WJ (2005) Synaptic vesicle pools. Nat Rev Neurosci 6:57–69.

Robin NC, Agoston Z, Biechele TL, James RG, Berndt JD, Moon RT (2014) Simvastatin Promotes Adult Hippocampal Neurogenesis by Enhancing Wnt/β-Catenin Signaling. Stem Cell Rep 2:9–17.

Rocha NP, de Miranda AS, Teixeira AL (2015) Insights into Neuroinflammation in Parkinson's Disease: From Biomarkers to Anti-Inflammatory Based Therapies. BioMed Res Int 2015:628192.

Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, Obeso JA (2009) Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. Lancet Neurol 8:1128–1139.

Rodriguez-Perez AI, Dominguez-Meijide A, Lanciego JL, Guerra MJ, Labandeira-Garcia JL (2013) Inhibition of Rho kinase mediates the neuroprotective effects of estrogen in the MPTP model of Parkinson's disease. Neurobiol Dis 58:209–219.

Roglans N, Verd JC, Peris C, Alegret M, Vazquez M, Adzet T, Diaz C, Hernandez G, Laguna JC, Sanchez RM (2002) High doses of atorvastatin and simvastatin induce key enzymes involved in VLDL production. Lipids 37:445–454.

Róg T, Pasenkiewicz-Gierula M, Vattulainen I, Karttunen M (2009) Ordering effects of cholesterol and its analogues. Biochim Biophys Acta 1788:97–121.

Roos BE, Steg G (1964) THE EFFECT OF L-3, 4-DIHYDROXYPHENYLALANINE AND DL-5-HYDROXYTRYPTOPHAN ON RIGIDITY AND TREMOR INDUCED BY RESERPINE, CHLORPROMAZINE AND PHENOXYBENZAMINE. Life Sci 3:351–360.

Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Björklund A (1999) Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. Eur J Neurosci 11:1554–1566.

Roth JA (1992) Membrane-bound catechol-O-methyltransferase: a reevaluation of its role in the O-methylation of the catecholamine neurotransmitters. Rev Physiol Biochem Pharmacol 120:1–29.

Roy A, Pahan K (2011) Prospects of statins in Parkinson disease. Neuroscientist 17:244–255.

Rudnick G, Steiner-Mordoch SS, Fishkes H, Stern-Bach Y, Schuldiner S (1990) Energetics of reserpine binding and occlusion by the chromaffin granule biogenic amine transporter. Biochemistry (Mosc) 29:603–608.

Ruiz-Martinez J, Krebs CE, Makarov V, Gorostidi A, Martí-Massó JF, Paisán-Ruiz C (2015) GIGYF2 mutation in late-onset Parkinson's disease with cognitive impairment. J Hum Genet.

Russell DW, Halford RW, Ramirez DMO, Shah R, Kotti T (2009) Cholesterol 24-hydroxylase: an enzyme of cholesterol turnover in the brain. Annu Rev Biochem 78:1017–1040.

Ryan BJ, Hoek S, Fon EA, Wade-Martins R (2015) Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. Trends Biochem Sci 40:200–210.

Saal K-A, Koch JC, Tatenhorst L, Szegő ÉM, Ribas VT, Michel U, Bähr M, Tönges L, Lingor P (2015) AAV.shRNA-mediated downregulation of ROCK2 attenuates degeneration of dopaminergic neurons in toxin-induced models of Parkinson's disease in vitro and in vivo. Neurobiol Dis 73:150–162.

Sacchetti P, Dwornik H, Formstecher P, Rachez C, Lefebvre P (2002) Requirements for heterodimerization between the orphan nuclear receptor Nurr1 and retinoid X receptors. J Biol Chem 277:35088–35096.

Sacchetti P, Sousa KM, Hall AC, Liste I, Steffensen KR, Theofilopoulos S, Parish CL, Hazenberg C, Richter LA, Hovatta O, Gustafsson JA, Arenas E (2009) Liver X receptors and oxysterols promote ventral midbrain neurogenesis in vivo and in human embryonic stem cells. Cell Stem Cell 5:409–419.

Saha AR, Hill J, Utton MA, Asuni AA, Ackerley S, Grierson AJ, Miller CC, Davies AM, Buchman VL, Anderton BH, Hanger DP (2004) Parkinson's disease alpha-synuclein mutations exhibit defective axonal transport in cultured neurons. J Cell Sci 117:1017–1024.

Saha N, Kolev M, Nikolov DB (2014) Structural features of the Nogo receptor signaling complexes at the neuron/myelin interface. Neurosci Res 87:1–7.

Saher G, Brügger B, Lappe-Siefke C, Möbius W, Tozawa R, Wehr MC, Wieland F, Ishibashi S, Nave K-A (2005) High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8:468–475.

Samuel F, Reddy J, Kaimal R, Segovia V, Mo H, Hynds DL (2014) Inhibiting geranylgeranylation increases neurite branching and differentially activates cofilin in cell bodies and growth cones. Mol Neurobiol 50:49–59.

Sandoval V, Riddle EL, Hanson GR, Fleckenstein AE (2002) Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. J Neurosci Off J Soc Neurosci 22:8705–8710.

Sano I, Gamo T, Kakimoto Y, Taniguchi K, Takesada M, Nishinuma K (1959) Distribution of catechol compounds in human brain. Biochim Biophys Acta 32:586–587.

Santiago M, Hernández-Romero MC, Machado A, Cano J (2009) Zocor Forte (simvastatin) has a neuroprotective effect against LPS striatal dopaminergic terminals injury, whereas against MPP+ does not. Eur J Pharmacol 609:58–64.

Santiago M, Matarredona ER, Granero L, Cano J, Machado A (2000) Neurotoxic relationship between dopamine and iron in the striatal dopaminergic nerve terminals. Brain Res 858:26–32.

Santpere G, Ferrer I (2009) LRRK2 and neurodegeneration. Acta Neuropathol (Berl) 117:227–246.

Satoh M, Takahashi Y, Tabuchi T, Minami Y, Tamada M, Takahashi K, Itoh T, Morino Y, Nakamura M (2015) Cellular and molecular mechanisms of statins: an update on pleiotropic effects. Clin Sci Lond Engl 1979 129:93–105.

Sato R (2009) SREBPs: protein interaction and SREBPs. FEBS J 276:622–627.

Sato R (2010) Sterol metabolism and SREBP activation. Arch Biochem Biophys 501:177–181.

Sato R, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M (1996) Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. J Biol Chem 271:26461–26464.

Sato-Suzuki I, Murota S (1996) Simvastatin inhibits the division and induces neurite-like outgrowth in PC12 cells. Neurosci Lett 220:21–24.

Sauer H, Oertel WH (1994) Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience 59:401–415.

Schafer WR, Rine J (1992) Protein prenylation: genes, enzymes, targets, and functions. Annu Rev Genet 26:209–237.

Scherman D, Henry JP (1982) Acido-basic properties of the catecholamine uptake inhibitors tetrabenazine and dihydrotetrabenazine. Biochimie 64:915–921.

Schlager MA, Hoogenraad CC (2009) Basic mechanisms for recognition and transport of synaptic cargos. Mol Brain 2:25.

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108.

Schmitt KC, Rothman RB, Reith MEA (2013) Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. J Pharmacol Exp Ther 346:2–10.

Schmitt M, Dehay B, Bezard E, Garcia-Ladona FJ (2015) Harnessing the Trophic and Modulatory Potential of Statins in a Dopaminergic Cell Line. Submitt Synap.

Schuldiner S, Liu Y, Edwards RH (1993) Reserpine binding to a vesicular amine transporter expressed in Chinese hamster ovary fibroblasts. J Biol Chem 268:29–34.

Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B (2000) Role of LXRs in control of lipogenesis. Genes Dev 14:2831–2838.

Schulz JG, Bosel J, Stoeckel M, Megow D, Dirnagl U, Endres M (2004) HMG-CoA reductase inhibition causes neurite loss by interfering with geranylgeranylpyrophosphate synthesis. J Neurochem 89:24–32.

Schütz B, Schäfer MK, Eiden LE, Weihe E (1998) Vesicular amine transporter expression and isoform selection in developing brain, peripheral nervous system and gut. Brain Res Dev Brain Res 106:181–204.

Schwarting RK, Huston JP (1996) The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 50:275–331.

Segatto M, Leboffe L, Trapani L, Pallottini V (2014) Cholesterol homeostasis failure in the brain: implications for synaptic dysfunction and cognitive decline. Curr Med Chem 21:2788–2802.

Segura-Aguilar J, Paris I, Munoz P, Ferrari E, Zecca L, Zucca FA (2014) Protective and toxic roles of dopamine in Parkinson's disease. J Neurochem 129:898–915.

Seiler S, Di Santo S, Widmer HR (2015) Non-canonical actions of Nogo-A and its receptors. Biochem Pharmacol.

Selley ML (2005) Simvastatin prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced striatal dopamine depletion and protein tyrosine nitration in mice. Brain Res 1037:1–6.

Sepulveda B, Mesias R, Li X, Yue Z, Benson DL (2013) Short- and long-term effects of LRRK2 on axon and dendrite growth. PloS One 8:e61986.

Serra PA, Sciola L, Delogu MR, Spano A, Monaco G, Miele E, Rocchitta G, Miele M, Migheli R, Desole MS (2002) The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induces apoptosis in mouse nigrostriatal glia. Relevance to nigral neuronal death and striatal neurochemical changes. J Biol Chem 277:34451–34461.

Shanware NP, Bray K, Abraham RT (2013) The PI3K, metabolic, and autophagy networks: interactive partners in cellular health and disease. Annu Rev Pharmacol Toxicol 53:89–106.

Shao W, Espenshade PJ (2012) Expanding roles for SREBP in metabolism. Cell Metab 16:414–419.

Shao W, Espenshade PJ (2014) Sterol regulatory element-binding protein (SREBP) cleavage regulates Golgi-to-endoplasmic reticulum recycling of SREBP cleavage-activating protein (SCAP). J Biol Chem 289:7547–7557.

Shimano H (2001) Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res 40:439–452.

Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N (1999) Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 274:35832–35839.

Shin ES, Lee HH, Cho SY, Park HW, Lee SJ, Lee TR (2007) Genistein downregulates SREBP-1 regulated gene expression by inhibiting site-1 protease expression in HepG2 cells. J Nutr 137:1127–1131.

Shojaee S, Sina F, Banihosseini SS, Kazemi MH, Kalhor R, Shahidi G-A, Fakhrai-Rad H, Ronaghi M, Elahi E (2008) Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. Am J Hum Genet 82:1375–1384.

Shults CW (2006) Lewy bodies. Proc Natl Acad Sci U S A 103:1661–1668.

Siderowf A, Kurlan R (1999) Monoamine oxidase and catechol-O-methyltransferase inhibitors. Med Clin North Am 83:445–467.

Sidhu A, Niznik HB (2000) Coupling of dopamine receptor subtypes to multiple and diverse G proteins. Int J Dev Neurosci Off J Int Soc Dev Neurosci 18:669–677.

Sidhu A, Wersinger C, Vernier P (2004a) alpha-Synuclein regulation of the dopaminergic transporter: a possible role in the pathogenesis of Parkinson's disease. FEBS Lett 565:1–5.

Sidhu A, Wersinger C, Vernier P (2004b) Does alpha-synuclein modulate dopaminergic synaptic content and tone at the synapse? FASEB J Off Publ Fed Am Soc Exp Biol 18:637–647.

Sierra S, Ramos MC, Molina P, Esteo C, Vázquez JA, Burgos JS (2011) Statins as neuroprotectants: a comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. J Alzheimers Dis JAD 23:307–318.

Siirtola T, Sonninen V, Rinne UK (1975) Urinary excretion of monoamines and their metabolites in patients with Parkinson's disease. Response to long-term treatment with levodopa alone or in combination with a dopa decarboxylase inhibitor and clinical correlations. Clin Neurol Neurosurg 78:77–88.

Sikorska B, Papierz W, Preusser M, Liberski PP, Budka H (2007) Synucleinopathy with features of both multiple system atrophy and dementia with Lewy bodies. Neuropathol Appl Neurobiol 33:126–129.

Silva T, Teixeira J, Remião F, Borges F (2013) Alzheimer's disease, cholesterol, and statins: the junctions of important metabolic pathways. Angew Chem Int Ed Engl 52:1110–1121.

Simola N, Morelli M, Carta AR (2007) The 6-hydroxydopamine model of Parkinson's disease. Neurotox Res 11:151–167.

Simon KC, Chen H, Schwarzschild M, Ascherio A (2007) Hypertension, hypercholesterolemia, diabetes, and risk of Parkinson disease. Neurology 69:1688–1695.

Simons K, Ehehalt R (2002) Cholesterol, lipid rafts, and disease. J Clin Invest 110:597–603.

Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–572.

Sirtori CR (2014) The pharmacology of statins. Pharmacol Res Off J Ital Pharmacol Soc 88:3–11.

Skerrett R, Malm T, Landreth G (2014) Nuclear receptors in neurodegenerative diseases. Neurobiol Dis 72 Pt A:104–116.

Slim HB, Black HR, Thompson PD (2011) Older blood pressure medications-do they still have a place? Am J Cardiol 108:308–316.

Smidt MP, Smits SM, Burbach JPH (2004) Homeobox gene Pitx3 and its role in the development of dopamine neurons of the substantia nigra. Cell Tissue Res 318:35–43.

Smith Y, Wichmann T, Factor SA, DeLong MR (2012) Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 37:213–246.

Smits SM, Ponnio T, Conneely OM, Burbach JPH, Smidt MP (2003) Involvement of Nurr1 in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. Eur J Neurosci 18:1731–1738.

Song G, Ouyang G, Bao S (2005) The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 9:59–71.

Sorkina T, Hoover BR, Zahniser NR, Sorkin A (2005) Constitutive and protein kinase C-induced internalization of the dopamine transporter is mediated by a clathrin-dependent mechanism. Traffic 6:157–170.

Sorkina T, Miranda M, Dionne KR, Hoover BR, Zahniser NR, Sorkin A (2006) RNA interference screen reveals an essential role of Nedd4-2 in dopamine transporter ubiquitination and endocytosis. J Neurosci Off J Soc Neurosci 26:8195–8205.

Sousa VL, Bellani S, Giannandrea M, Yousuf M, Valtorta F, Meldolesi J, Chieregatti E (2009) {alpha}-synuclein and its A30P mutant affect actin cytoskeletal structure and dynamics. Mol Biol Cell 20:3725–3739.

Spacek J, Harris KM (1998) Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus. J Comp Neurol 393:58–68.

Sparrow SM, Carter JM, Ridgway ND, Cook HW, Byers DM (1999) U18666A inhibits intracellular cholesterol transport and neurotransmitter release in human neuroblastoma cells. Neurochem Res 24:69–77.

Speciale SG, Liang CL, Sonsalla PK, Edwards RH, German DC (1998) The neurotoxin 1-methyl-4-phenylpyridinium is sequestered within neurons that contain the vesicular monoamine transporter. Neuroscience 84:1177–1185.

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998) Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J Neurochem 71:2112–2122.

Spencer PS (2000) Mechanisms of Selective Toxicity in Neurodegenerative Disorders. In: Neurotoxic Factors in Parkinson's Disease and Related Disorders (Storch A, Collins MA, eds), pp 1–16. Boston, MA: Springer US. Available at: http://link.springer.com/10.1007/978-1-4615-1269-1_1 [Accessed September 16, 2015].

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95:6469–6473.

Staal RG, Hogan KA, Liang CL, German DC, Sonsalla PK (2000) In vitro studies of striatal vesicles containing the vesicular monoamine transporter (VMAT2): rat versus mouse differences in sequestration of 1-methyl-4-phenylpyridinium. J Pharmacol Exp Ther 293:329–335.

Staal RG, Sonsalla PK (2000) Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. J Pharmacol Exp Ther 293:336–342.

Stanley-Jones D (1956) The anatomy of rigidity and tremor. J Nerv Ment Dis 124:163–166.

Stayte S, Vissel B (2014) Advances in non-dopaminergic treatments for Parkinson's disease. Front Neurosci 8:113.

Steiner H, Tseng K-Y (2010) Handbook of Basal Ganglia Structure and Function. Burlington: Elsevier. Available at: http://public.eblib.com/choice/publicfullrecord.aspx?p=535020 [Accessed October 1, 2015].

Stein M-P, Dong J, Wandinger-Ness A (2003) Rab proteins and endocytic trafficking: potential targets for therapeutic intervention. Adv Drug Deliv Rev 55:1421–1437.

Stelmashook EV, Isaev NK, Genrikhs EE, Amelkina GA, Khaspekov LG, Skrebitsky VG, Illarioshkin SN (2014) Role of zinc and copper ions in the pathogenetic mechanisms of Alzheimer's and Parkinson's diseases. Biochem Biokhimiia 79:391–396.

Stephenson DT, Childs MA, Li Q, Carvajal-Gonzalez S, Opsahl A, Tengowski M, Meglasson MD, Merchant K, Emborg ME (2007) Differential loss of presynaptic dopaminergic markers in Parkinsonian monkeys. Cell Transplant 16:229–244.

Storch A, Ludolph AC, Schwarz J (1999) HEK-293 cells expressing the human dopamine transporter are susceptible to low concentrations of 1-methyl-4-phenylpyridine (MPP+) via impairment of energy metabolism. Neurochem Int 35:393–403.

Storch A, Ott S, Hwang YI, Ortmann R, Hein A, Frenzel S, Matsubara K, Ohta S, Wolf HU, Schwarz J (2002) Selective dopaminergic neurotoxicity of isoquinoline derivatives related to Parkinson's disease: studies using heterologous expression systems of the dopamine transporter. Biochem Pharmacol 63:909–920.

Street CA, Bryan BA (2011) Rho kinase proteins--pleiotropic modulators of cell survival and apoptosis. Anticancer Res 31:3645–3657.

Surgucheva I, He S, Rich MC, Sharma R, Ninkina NN, Stahel PF, Surguchov A (2014) Role of synucleins in traumatic brain injury — an experimental in vitro and in vivo study in mice. Mol Cell Neurosci 63:114–123.

Surmeier DJ, Song WJ, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci Off J Soc Neurosci 16:6579–6591.

Suzuki R, Lee K, Jing E, Biddinger SB, McDonald JG, Montine TJ, Craft S, Kahn CR (2010) Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism. Cell Metab 12:567–579.

Svenningsson P, Nishi A, Fisone G, Girault J-A, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol 44:269–296.

Swant J, Goodwin JS, North A, Ali AA, Gamble-George J, Chirwa S, Khoshbouei H (2011) α-Synuclein stimulates a dopamine transporter-dependent chloride current and modulates the activity of the transporter. J Biol Chem 286:43933–43943.

Tabernero L, Rodwell VW, Stauffacher CV (2003) Crystal structure of a statin bound to a class II hydroxymethylglutaryl-CoA reductase. J Biol Chem 278:19933–19938.

Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR (1997) VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci 94:9938–9943.

Takamori S et al. (2006) Molecular anatomy of a trafficking organelle. Cell 127:831–846.

Takemoto M, Liao JK (2001) Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. Arterioscler Thromb Vasc Biol 21:1712–1719.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW (1999) Parkinson disease in twins: an etiologic study. JAMA 281:341–346.

Tarsa L, Goda Y (2002) Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons. Proc Natl Acad Sci U S A 99:1012–1016.

Tarsy D, Vitek JL, Lozano AM (2003) Surgical treatment of Parkinson's disease and other movement disorders. Totowa, N.J.: Humana. Available at: http://site.ebrary.com/id/10181432 [Accessed October 1, 2015].

Taylor F, Huffman MD, Macedo AF, Moore THM, Burke M, Davey Smith G, Ward K, Ebrahim S (2013) Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 1:CD004816.

Taylor TN, Caudle WM, Miller GW (2011) VMAT2-Deficient Mice Display Nigral and Extranigral Pathology and Motor and Nonmotor Symptoms of Parkinson's Disease. Park Dis 2011:124165.

Tepper JM, Bolam JP (2004) Functional diversity and specificity of neostriatal interneurons. Curr Opin Neurobiol 14:685–692.

Theofilopoulos S, Wang Y, Kitambi SS, Sacchetti P, Sousa KM, Bodin K, Kirk J, Salto C, Gustafsson M, Toledo EM, Karu K, Gustafsson JA, Steffensen KR, Ernfors P, Sjovall J, Griffiths WJ, Arenas E (2013) Brain endogenous liver X receptor ligands selectively promote midbrain neurogenesis. Nat Chem Biol 9:126–133.

Thiele C, Hannah MJ, Fahrenholz F, Huttner WB (2000) Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nat Cell Biol 2:42–49.

Thiriez C, Villafane G, Grapin F, Fenelon G, Remy P, Cesaro P (2011) Can nicotine be used medicinally in Parkinson's disease? Expert Rev Clin Pharmacol 4:429–436.

Tian L, Karimi M, Loftin SK, Brown CA, Xia H, Xu J, Mach RH, Perlmutter JS (2012) No differential regulation of dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) binding in a primate model of Parkinson disease. PloS One 7:e31439.

Tieu K (2011) A guide to neurotoxic animal models of Parkinson's disease. Cold Spring Harb Perspect Med 1:a009316.

Tieu K, Ischiropoulos H, Przedborski S (2003) Nitric oxide and reactive oxygen species in Parkinson's disease. IUBMB Life 55:329–335.

Tobert JA (2003) Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nat Rev Drug Discov 2:517–526.

Tolwani RJ, Jakowec MW, Petzinger GM, Green S, Waggie K (1999) Experimental models of Parkinson's disease: insights from many models. Lab Anim Sci 49:363–371.

Tonges L, Frank T, Tatenhorst L, Saal KA, Koch JC, Szego EM, Bahr M, Weishaupt JH, Lingor P (2012) Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease. Brain 135:3355–3370.

Tönges L, Koch J-C, Bähr M, Lingor P (2011) ROCKing Regeneration: Rho Kinase Inhibition as Molecular Target for Neurorestoration. Front Mol Neurosci 4:39.

Tönges L, Szegö EM, Hause P, Saal K-A, Tatenhorst L, Koch JC, D Hedouville Z, Dambeck V, Kügler S, Dohm CP, Bähr M, Lingor P (2014) Alpha-synuclein mutations impair axonal regeneration in models of Parkinson's disease. Front Aging Neurosci 6:239.

Tong J, Boileau I, Furukawa Y, Chang LJ, Wilson AA, Houle S, Kish SJ (2011) Distribution of vesicular monoamine transporter 2 protein in human brain: implications for brain imaging studies. J Cereb Blood Flow Metab 31:2065–2075.

Torres GE (2006) The dopamine transporter proteome. J Neurochem 97 Suppl 1:3–10.

Torres GE, Carneiro A, Seamans K, Fiorentini C, Sweeney A, Yao W-D, Caron MG (2003a) Oligomerization and trafficking of the human dopamine transporter. Mutational analysis identifies critical domains important for the functional expression of the transporter. J Biol Chem 278:2731–2739.

Torres GE, Gainetdinov RR, Caron MG (2003b) Plasma membrane monoamine transporters: structure, regulation and function. Nat Rev Neurosci 4:13–25.

Truong JG, Newman AH, Hanson GR, Fleckenstein AE (2004) Dopamine D2 receptor activation increases vesicular dopamine uptake and redistributes vesicular monoamine transporter-2 protein. Eur J Pharmacol 504:27–32.

Tsika E, Glauser L, Moser R, Fiser A, Daniel G, Sheerin U-M, Lees A, Troncoso JC, Lewis PA, Bandopadhyay R, Schneider BL, Moore DJ (2014) Parkinson's disease-linked mutations in VPS35 induce dopaminergic neurodegeneration. Hum Mol Genet 23:4621–4638.

Tsika E, Nguyen APT, Dusonchet J, Colin P, Schneider BL, Moore DJ (2015) Adenoviral-mediated expression of G2019S LRRK2 induces striatal pathology in a kinase-dependent manner in a rat model of Parkinson's disease. Neurobiol Dis 77:49–61.

Ugolev Y, Segal T, Yaffe D, Gros Y, Schuldiner S (2013) Identification of conformationally sensitive residues essential for inhibition of vesicular monoamine transport by the noncompetitive inhibitor tetrabenazine. J Biol Chem 288:32160–32171.

Uhl GR (2003) Dopamine transporter: basic science and human variation of a key molecule for dopaminergic function, locomotion, and parkinsonism. Mov Disord Off J Mov Disord Soc 18 Suppl 7:S71–S80.

Uhl GR, Walther D, Mash D, Faucheux B, Javoy-Agid F (1994) Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons. Ann Neurol 35:494–498.

Ulusoy GK, Celik T, Kayir H, Gürsoy M, Isik AT, Uzbay TI (2011) Effects of pioglitazone and retinoic acid in a rotenone model of Parkinson's disease. Brain Res Bull 85:380–384.

Undela K, Gudala K, Malla S, Bansal D (2013) Statin use and risk of Parkinson's disease: a meta-analysis of observational studies. J Neurol 260:158–165.

Ungerstedt U (1968) 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110.

Uranga RM, Katz S, Salvador GA (2013) Enhanced phosphatidylinositol 3-kinase (PI3K)/Akt signaling has pleiotropic targets in hippocampal neurons exposed to iron-induced oxidative stress. J Biol Chem 288:19773–19784.

Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7:65–74.

Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, Albanese A, Wood NW (2001) Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 68:895–900.

Vallone D, Picetti R, Borrelli E (2000) Structure and function of dopamine receptors. Neurosci Biobehav Rev 24:125–132.

Van Den Eeden SK, Tanner CM, Bernstein AL, Fross RD, Leimpeter A, Bloch DA, Nelson LM (2003) Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol 157:1015–1022.

van den Munckhof P, Luk KC, Ste-Marie L, Montgomery J, Blanchet PJ, Sadikot AF, Drouin J (2003) Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. Dev Camb Engl 130:2535–2542.

van der Most PJ, Dolga AM, Nijholt IM, Luiten PG, Eisel UL (2009) Statins: mechanisms of neuroprotection. Prog Neurobiol 88:64–75.

van Duijn CM, Dekker MC, Bonifati V, Galjaard RJ, Houwing-Duistermaat JJ, Snijders PJ, Testers L, Breedveld GJ, Horstink M, Sandkuijl LA, van Swieten JC, Oostra BA, Heutink P (2001) Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am J Hum Genet 69:629–634.

Vaughan RA, Brown VL, McCoy MT, Kuhar MJ (1996) Species- and brain region-specific dopamine transporters: immunological and glycosylation characteristics. J Neurochem 66:2146–2152.

Vaughan RA, Foster JD (2013) Mechanisms of dopamine transporter regulation in normal and disease states. Trends Pharmacol Sci 34:489–496.

Vaughan RA, Huff RA, Uhl GR, Kuhar MJ (1997) Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes. J Biol Chem 272:15541–15546.

Vaya J, Schipper HM (2007) Oxysterols, cholesterol homeostasis, and Alzheimer disease. J Neurochem 102:1727–1737.

Vergo S, Johansen JL, Leist M, Lotharius J (2007) Vesicular monoamine transporter 2 regulates the sensitivity of rat dopaminergic neurons to disturbed cytosolic dopamine levels. Brain Res 1185:18–32.

Vilariño-Güell C et al. (2014) DNAJC13 mutations in Parkinson disease. Hum Mol Genet 23:1794–1801.

Volkmann J (2004) Deep brain stimulation for the treatment of Parkinson's disease. J Clin Neurophysiol Off Publ Am Electroencephalogr Soc 21:6–17.

Volknandt W, Karas M (2012) Proteomic analysis of the presynaptic active zone. Exp Brain Res 217:449–461.

Volles MJ, Lansbury PT (2002) Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. Biochemistry (Mosc) 41:4595–4602.

Volles MJ, Lansbury PT (2003) Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. Biochemistry (Mosc) 42:7871–7878.

Volz TJ (2008) Neuropharmacological mechanisms underlying the neuroprotective effects of methylphenidate. Curr Neuropharmacol 6:379.

Volz TJ, Farnsworth SJ, Hanson GR, Fleckenstein AE (2009) Method development and validation of an in vitro model of the effects of methylphenidate on membrane-associated synaptic vesicles. J Neurosci Methods 177:177–182.

von Holst A, Rodriguez-Tébar A, Michaille JJ, Dhouailly D, Bäckström A, Ebendal T, Rohrer H (1995) Retinoic acid-mediated increase in TrkA expression is sufficient to elicit NGF-dependent survival of sympathetic neurons. Mol Cell Neurosci 6:185–198.

Wahner AD, Bronstein JM, Bordelon YM, Ritz B (2008) Statin use and the risk of Parkinson disease. Neurology 70:1418–1422.

Waites CL, Mehta A, Tan PK, Thomas G, Edwards RH, Krantz DE (2001) An acidic motif retains vesicular monoamine transporter 2 on large dense core vesicles. J Cell Biol 152:1159–1168.

Wakabayashi K, Tanji K, Mori F, Takahashi H (2007) The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. Neuropathol Off J Jpn Soc Neuropathol 27:494–506.

Wallen-Mackenzie A, Mata de Urquiza A, Petersson S, Rodriguez FJ, Friling S, Wagner J, Ordentlich P, Lengqvist J, Heyman RA, Arenas E, Perlmann T (2003) Nurr1-RXR heterodimers mediate RXR ligand-induced signaling in neuronal cells. Genes Dev 17:3036–3047.

Wang G, Pan J, Chen S-D (2012) Kinases and kinase signaling pathways: potential therapeutic targets in Parkinson's disease. Prog Neurobiol 98:207–221.

Wang L, Schuster GU, Hultenby K, Zhang Q, Andersson S, Gustafsson J-A (2002) Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. Proc Natl Acad Sci U S A 99:13878–13883.

Wang M, Pei L, Fletcher PJ, Kapur S, Seeman P, Liu F (2010) Schizophrenia, amphetamine-induced sensitized state and acute amphetamine exposure all show a common alteration: increased dopamine D2 receptor dimerization. Mol Brain 3:25.

Wang PY, Seabold GK, Wenthold RJ (2008) Synaptic adhesion-like molecules (SALMs) promote neurite outgrowth. Mol Cell Neurosci 39:83–94.

Wang Q, Tang XN, Wang L, Yenari MA, Ying W, Goh B-C, Lee H-S, Wilder-Smith EP, Wong PT (2006) Effects of high dose of simvastatin on levels of dopamine and its reuptake in prefrontal cortex and striatum among SD rats. Neurosci Lett 408:189–193.

Wang Q, Ting WL, Yang H, Wong PT-H (2005) High doses of simvastatin upregulate dopamine D1 and D2 receptor expression in the rat prefrontal cortex: possible involvement of endothelial nitric oxide synthase. Br J Pharmacol 144:933–939.

Wang Q, Yan J, Chen X, Li J, Yang Y, Weng J, Deng C, Yenari MA (2011) Statins: multiple neuroprotective mechanisms in neurodegenerative diseases. Exp Neurol 230:27–34.

Wang X, Sato R, Brown MS, Hua X, Goldstein JL (1994) SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell 77:53–62.

Wang YM, Gainetdinov RR, Fumagalli F, Xu F, Jones SR, Bock CB, Miller GW, Wightman RM, Caron MG (1997) Knockout of the vesicular monoamine transporter 2 gene results in neonatal death and supersensitivity to cocaine and amphetamine. Neuron 19:1285–1296.

Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Nishikawa S, Muguruma K, Sasai Y (2007) A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol 25:681–686.

Watanabe T, Yasutaka Y, Nishioku T, Nakashima A, Futagami K, Yamauchi A, Kataoka Y (2012) Atorvastatin stimulates neuroblastoma cells to induce neurite outgrowth by increasing cellular prion protein expression. Neurosci Lett 531:114–119.

Waxman EA, Giasson BI (2009) Molecular mechanisms of alpha-synuclein neurodegeneration. Biochim Biophys Acta 1792:616–624.

Weihe E, Schäfer MK, Erickson JD, Eiden LE (1994) Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. J Mol Neurosci MN 5:149–164.

Wei Q, Wang H, Tian Y, Xu F, Chen X, Wang K (2013) Reduced serum levels of triglyceride, very low density lipoprotein cholesterol and apolipoprotein B in Parkinson's disease patients. PloS One 8:e75743.

Wersinger C, Prou D, Vernier P, Sidhu A (2003) Modulation of dopamine transporter function by alpha-synuclein is altered by impairment of cell adhesion and by induction of oxidative stress. FASEB J Off Publ Fed Am Soc Exp Biol 17:2151–2153.

Wersinger C, Sidhu A (2003) Attenuation of dopamine transporter activity by alpha-synuclein. Neurosci Lett 340:189–192.

Whitton PS (2007) Inflammation as a causative factor in the aetiology of Parkinson's disease. Br J Pharmacol 150:963–976.

Willey JZ, Elkind MS (2010) 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in the treatment of central nervous system diseases. Arch Neurol 67:1062–1067.

Wilson (1925) THE Croonian Lectures ON SOME DISORDERS OF MOTILITY AND OF MUSCLE TONE, WITH SPECIAL REFERENCE TO THE CORPUS STRIATUM. The Lancet 206:215–219.

Wilson JD (1972) The relation between cholesterol absorption and cholesterol synthesis in the baboon. J Clin Invest 51:1450–1458.

Wilson JD, Lindsey CA (1965) Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man. J Clin Invest 44:1805–1814.

Wilson MD, Rudel LL (1994) Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. J Lipid Res 35:943–955.

Wimalasena K (2011) Vesicular monoamine transporters: structure-function, pharmacology, and medicinal chemistry. Med Res Rev 31:483–519.

Winkler S, Vollstedt E-J, Kasten M, Alvarez-Fischer D, Klein C, Lohmann K (2014) The recurrent mutation Arg258Gln in SYNJ1 (PARK20) is not a common cause of Parkinson's disease. J Neurol 261:833–834.

Winner B, Melrose HL, Zhao C, Hinkle KM, Yue M, Kent C, Braithwaite AT, Ogholikhan S, Aigner R, Winkler J (2011) Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. Neurobiol Dis 41:706–716.

Winter S, Brunk I, Walther DJ, Höltje M, Jiang M, Peter J-U, Takamori S, Jahn R, Birnbaumer L, Ahnert-Hilger G (2005) Galphao2 regulates vesicular glutamate transporter activity by changing its chloride dependence. J Neurosci Off J Soc Neurosci 25:4672–4680.

Wishart S, Macphee GJA (2011) Evaluation and management of the non-motor features of Parkinson's disease. Ther Adv Chronic Dis 2:69–85.

Wolozin B, Wang SW, Li NC, Lee A, Lee TA, Kazis LE (2007) Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. BMC Med 5:20.

Wolters EC, Baumann CR, International Association of Parkinsonism and Related Disorders (2014) Parkinson disease and other movement disorders: motor behavioural disorders and behavioural motor disorders. Amsterdam, The Netherlands; Chicago: International Association of Parkinsonism and Related Disorders : VU University Press ; Distributed by Independent Publishers Group.

Wood WG, Műller WE, Eckert GP (2014) Statins and neuroprotection: basic pharmacology needed. Mol Neurobiol 50:214–220.

Worgall TS, Johnson RA, Seo T, Gierens H, Deckelbaum RJ (2002) Unsaturated fatty acid-mediated decreases in sterol regulatory element-mediated gene transcription are linked to cellular sphingolipid metabolism. J Biol Chem 277:3878–3885.

Wu H, Lu D, Jiang H, Xiong Y, Qu C, Li B, Mahmood A, Zhou D, Chopp M (2008) Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. J Neurotrauma 25:130–139.

Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, Conway SJ, Ganapathy V (1998) Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J Biol Chem 273:32776–32786.

Xiao X, Song B-L (2013) SREBP: a novel therapeutic target. Acta Biochim Biophys Sin 45:2–10.

Xu P, Xu H, Tang X, Xu L, Wang Y, Guo L, Yang Z, Xing Y, Wu Y, Warner M, Gustafsson J-A, Fan X (2014) Liver X receptor β is essential for the differentiation of radial glial cells to oligodendrocytes in the dorsal cortex. Mol Psychiatry 19:947–957.

Xu T, Bajjalieh SM (2001) SV2 modulates the size of the readily releasable pool of secretory vesicles. Nat Cell Biol 3:691–698.

Xu YQ, Long L, Yan JQ, Wei L, Pan MQ, Gao HM, Zhou P, Liu M, Zhu CS, Tang BS, Wang Q (2013) Simvastatin induces neuroprotection in 6-OHDA-lesioned PC12 via the PI3K/AKT/caspase 3 pathway and anti-inflammatory responses. CNS Neurosci Ther 19:170–177.

Yabe D, Brown MS, Goldstein JL (2002) Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. Proc Natl Acad Sci U S A 99:12753–12758.

Yacoubian TA, Standaert DG (2009) Targets for neuroprotection in Parkinson's disease. Biochim Biophys Acta 1792:676–687.

Yang D, Han Y, Zhang J, Chopp M, Seyfried DM (2012) Statins Enhance Expression of Growth Factors and Activate the PI3K/Akt-mediated Signaling Pathway after Experimental Intracerebral Hemorrhage. World J Neurosci 2:74–80.

Yang J, Goldstein JL, Hammer RE, Moon YA, Brown MS, Horton JD (2001) Decreased lipid synthesis in livers of mice with disrupted Site-1 protease gene. Proc Natl Acad Sci U S A 98:13607–13612.

Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E, Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature 393:809–812.

Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS (2002) Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell 110:489–500.

Yang Z-Z, Tschopp O, Baudry A, Dümmler B, Hynx D, Hemmings BA (2004) Physiological functions of protein kinase B/Akt. Biochem Soc Trans 32:350–354.

Yan J-Q, Ma Y-J, Sun J-C, Bai S-F, Huang L-N (2014a) Neuroprotective effect of lovastatin by inhibiting NMDA receptor1 in 6-hydroxydopamine treated PC12 cells. Int J Clin Exp Med 7:3313–3319.

Yan J, Sun J, Huang L, Fu Q, Du G (2014b) Simvastatin prevents neuroinflammation by inhibiting N-methyl-D-aspartic acid receptor 1 in 6-hydroxydopamine-treated PC12 cells. J Neurosci Res 92:634–640.

Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, Xiong Z, Deng C, Huang XF, Yenari MA, Yang YG, Ying W, Wang Q (2011) Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. PLoS One 6:e20945.

Yao J, Erickson JD, Hersh LB (2004) Protein kinase A affects trafficking of the vesicular monoamine transporters in PC12 cells. Traffic 5:1006–1016.

Yao J, Hersh LB (2007) The vesicular monoamine transporter 2 contains trafficking signals in both its N-glycosylation and C-terminal domains. J Neurochem 100:1387–1396.

Yeagle PL (1991) Modulation of membrane function by cholesterol. Biochimie 73:1303–1310.

Ye J, DeBose-Boyd RA (2011) Regulation of cholesterol and fatty acid synthesis. Cold Spring Harb Perspect Biol 3.

Yin L-H, Shen H, Diaz-Ruiz O, Bäckman CM, Bae E, Yu S-J, Wang Y (2012) Early post-treatment with 9-cis retinoic acid reduces neurodegeneration of dopaminergic neurons in a rat model of Parkinson's disease. BMC Neurosci 13:120.

Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL, Brown MS (1993) SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 75:187–197.

Yokoyama H, Yano R, Aoki E, Kato H, Araki T (2008) Comparative pharmacological study of free radical scavenger, nitric oxide synthase inhibitor, nitric oxide synthase activator and cyclooxygenase inhibitor against MPTP neurotoxicity in mice. Metab Brain Dis 23:335–349.

Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada N (2001) Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 21:2991–3000.

Youdim MBH, Bakhle YS (2006) Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br J Pharmacol 147 Suppl 1:S287–S296.

Yulug B, Hanoglu L, Kilic E (2015) The neuroprotective role of vesicular monoamine transporter 2 in neurodegenerative diseases. Med Chem 11:104–108.

Zacco A, Togo J, Spence K, Ellis A, Lloyd D, Furlong S, Piser T (2003) 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. J Neurosci Off J Soc Neurosci 23:11104–11111.

Zallakian M, Knoth J, Metropoulos GE, Njus D (1982) Multiple effects of reserpine on chromaffin-granule in membranes. Biochemistry (Mosc) 21:1051–1055.

Zarow C, Lyness SA, Mortimer JA, Chui HC (2003) Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. Arch Neurol 60:337–341.

Zecca L, Tampellini D, Gerlach M, Riederer P, Fariello RG, Sulzer D (2001) Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour. Mol Pathol MP 54:414–418.

Zecca L, Zucca FA, Wilms H, Sulzer D (2003) Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends Neurosci 26:578–580.

Zetterström RH, Solomin L, Mitsiadis T, Olson L, Perlmann T (1996) Retinoid X receptor heterodimerization and developmental expression distinguish the orphan nuclear receptors NGFI-B, Nurr1, and Nor1. Mol Endocrinol Baltim Md 10:1656–1666.

Zhang FL, Casey PJ (1996) Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241–269.

Zhang J, Dudley-Rucker N, Crowley JR, Lopez-Perez E, Issandou M, Schaffer JE, Ory DS (2004) The steroidal analog GW707 activates the SREBP pathway through disruption of intracellular cholesterol trafficking. J Lipid Res 45:223–231.

Zhang J, Kravtsov V, Amarnath V, Picklo MJ, Graham DG, Montine TJ (2000) Enhancement of dopaminergic neurotoxicity by the mercapturate of dopamine: relevance to Parkinson's disease. J Neurochem 74:970–978.

Zhang J, Liu Q (2015) Cholesterol metabolism and homeostasis in the brain. Protein Cell 6:254–264.

Zhang L, Zhang ZG, Liu XS, Hozeska-Solgot A, Chopp M (2007) The PI3K/Akt pathway mediates the neuroprotective effect of atorvastatin in extending thrombolytic therapy after embolic stroke in the rat. Arterioscler Thromb Vasc Biol 27:2470–2475.

Zhang Z, Ottens AK, Larner SF, Kobeissy FH, Williams ML, Hayes RL, Wang KKW (2006) Direct Rho-associated kinase inhibition [correction of inhibiton] induces cofilin dephosphorylation and neurite outgrowth in PC-12 cells. Cell Mol Biol Lett 11:12–29.

Zhao YJ, Wee HL, Chan Y-H, Seah SH, Au WL, Lau PN, Pica EC, Li SC, Luo N, Tan LCS (2010) Progression of Parkinson's disease as evaluated by Hoehn and Yahr stage transition times. Mov Disord Off J Mov Disord Soc 25:710–716.

Zhao Y, Zhang Q, Xi J, Li Y, Ma C, Xiao B (2015) Multitarget intervention of Fasudil in the neuroprotection of dopaminergic neurons in MPTP-mouse model of Parkinson's disease. J Neurol Sci 353:28–37.

Zheng G, Dwoskin LP, Crooks PA (2006) Vesicular monoamine transporter 2: role as a novel target for drug development. AAPS J 8:E682–E692.

Zheng Z, Chen B (2007) Effects of Pravastatin on neuroprotection and neurogenesis after cerebral ischemia in rats. Neurosci Bull 23:189–197.

Zhu M, Li J, Fink AL (2003) The association of alpha-synuclein with membranes affects bilayer structure, stability, and fibril formation. J Biol Chem 278:40186–40197.

Zhu SJ, Kavanaugh MP, Sonders MS, Amara SG, Zahniser NR (1997) Activation of protein kinase C inhibits uptake, currents and binding associated with the human dopamine transporter expressed in Xenopus oocytes. J Pharmacol Exp Ther 282:1358–1365.

Zou L, Jankovic J, Rowe DB, Xie W, Appel SH, Le W (1999) Neuroprotection by pramipexole against dopamine- and levodopa-induced cytotoxicity. Life Sci 64:1275–1285.